

Figure 1

GATCAAACCTCTTTTCCATTCAGAGTCCTCTGATTCAGATTTTAATGTAAACATTTTGAAGACAGTATTCAGAAAAAAATTTCC
TTAATAAAAAATACAACCTCAGATCCTTCAAATATGAACTGGTTGGGAATCTCCATTTTTTCAATATTATTTCTTCTTTGTTTTT
TTGCTACGTATAATTATTAATATCCTGACTAGGTTGTGGTTGGAGGGTTATTACTTTTCATTTTACCATGCAGTCCAAATCTAAAC
TGCTTCTACTGATGGTTTACAGCATTCTGAGATAAGAATGGTACATCTAGAGAACATTGCCAAAGGCCTAAGCACAGCAAAGGAA
AATAAACACAGAATATAATAAAATGAGATAATCTAGCTTAAACTATAACTTCCTCTTTAGAACTCCAACCACATTGGATC

FIG. 2A

5' 9 18 27 36 45 54
CAG AGA GGC TGT ATT TCA GTG CAG CCT GCC AGA CCT CTT CTG GAG GAA GAC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
63 72 81 90 99 108
ACA AAG GGG GTC ACA CAT TCC TTC CAT ACG GTT GAG CCT CTA CCT GCC TGG TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
117 126 135 144 153 162
TGG TCA CAG TTC AGC TTC ATG ATG GTG GAT CCC AAT GGC AAT GAA TCC AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
M M V D P N G N E S S
171 180 189 198 207 216
GCT ACA TAC TTC ATC CTA ATA GGC CTC CCT GGT TTA GAA GAG GCT CAG TTC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A T Y F I L I G L P G L E E A Q F W
225 234 243 252 261 270
TTG GCC TTC CCA TTG TGC TCC CTC TAC CTT ATT GCT GTG CTA GGT AAC TTG ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
L A F P L C S L Y L I A V L G N L T
279 288 297 306 315 324
ATC ATC TAC ATT GTG CGG ACT GAG CAC AGC CTG CAT GAG CCC ATG TAT ATA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
I I Y I V R T E H S L H E P M Y I F
333 342 351 360 369 378
CTT TGC ATG CTT TCA GGC ATT GAC ATC CTC ATC TCC ACC TCA TCC ATG CCC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
L C M L S G I D I L I S T S S M P K
387 396 405 414 423 432
ATG CTG GCC ATC TTC TGG TTC AAT TCC ACT ACC ATC CAG TTT GAT GCT TGT CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
M L A I F W F N S T T I Q F D A C L
441 450 459 468 477 486
CTA CAG ATT TTT GCC ATC CAC TCC TTA TCT GGC ATG GAA TCC ACA GTG CTG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
L Q I F A I H S L S G M E S T V L L
495 504 513 522 531 540
GCC ATG GCT TTT GAC CGC TAT GTG GCC ATC TGT CAC CCA CTG CGC CAT GCC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A M A F D R Y V A I C H P L R H A T
549 558 567 576 585 594
GTA CTT ACG TTG CCT CGT GTC ACC AAA ATT GGT GTG GCT GCT GTG GTG CGG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
V L T L P R V T K I G V A A V V R G
603 612 621 630 639 648
GCT GCA CTG ATG GCA CCC CTT CCT GTC TTC ATC AAG CAG CTG CCC TTC TGC CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A A L M A P L P V F I K Q L P F C R

FIG. 2B

657	666	675	684	693	702
TCC AAT ATC CTT	TCC CAT TCC TAC	TGC CTA CAC CAA	GAT GTC ATG AAG	CTG GCC	
S N I L	S H S Y	C L H Q	D V M K	L A	
711	720	729	738	747	756
TGT GAT GAT ATC	CGG GTC AAT GTC	TAT GGC CTT ATC	GTC ATC ATC TCC	GCC	
C D D I	R V N V	Y G L I	V I I S	A	
765	774	783	792	801	810
ATT GGC CTG GAC	TCA CTT CTC ATC	TCC TTC TCA TAT	CTG CTT ATT CTT	AAG ACT	
I G L D	S L L I	S F S Y	L L I L	K T	
819	828	837	846	855	864
GTG TTG GGC TTG	ACA CGT GAA GCC	CAG GCC AAG GCA	TTT GGC ACT TGC	GTC TCT	
V L G L	T R E A	Q A K A	F G T C	V S	
873	882	891	900	909	918
CAT GTG TGT GCT	GTG TTC ATA TTC	TAT GTA CCT TTC	ATT GGA TTG TCC	ATG GTG	
H V C A	V F I F	Y V P F	I G L S	M V	
927	936	945	954	963	972
CAT CGC TTT AGC	AAG CGG CGT GAC	TCT CCG CTG CCC	GTC ATC TTG GCC	AAT ATC	
H R F S	K R R D	S P L P	V I L A	N I	
981	990	999	1008	1017	1026
TAT CTG CTG GTT	CCT CCT GTG CTC	AAC CCA ATT GTC	TAT GGA GTG AAG	ACA AAG	
Y L L V	P P V L	N P I V	Y G V K	T K	
1035	1044	1053	1062	1071	1080
GAG ATT CGA CAG	CGC ATC CTT CGA	CTT TTC CAT GTG	GCC ACA CAC GCT	TCA GAG	
E I R Q	R I L R	L F H V	A T H A	S E	
1089	1098	1107	1116	1125	1134
CCC TAG GTG TCA	GTG ATC AAA CTT	CTT TTC CAT TCA	GAG TCC TCT GAT	TCA GAT	
P *					
1143	1152	1161	1170	1179	1188
TTT AAT GTT AAC	ATT TTG GAA GAC	AGT ATT CAG AAA	AAA AAT TTC CTT	AAT AAA	
1197	1206	1215	1224	1233	1242
AAA TAC AAC TCA	GAT CCT TCA AAT	ATG AAA CTG GTT	GGG GAA TCT CCA	TTT TTT	
1251	1260	1269	1278	1287	1296
CAA TAT TAT TTT	CTT CTT TGT TTT	CTT GCT ACA TAT	AAT TAT TAA TAC	CCT GAC	
1305	1314	1323	1332	1341	1350
TAG GTT GTG GTT	GGA GGG TTA TTA	CTT TTC ATT TTA	CCA TGC AGT CCA	AAT CTA	

FIG. 2C

1359	1368	1377	1386	1395	1404
AAC TGC TTC	TAC TGA TGG	TTT ACA GCA	TTC TGA GAT	AAG AAT GGT	ACA TCT AGA
1413	1422	1431	1440	1449	1458
GAA CAT TTG	CCA AAG GCC	TAA GCA CGG	CAA AGG AAA	ATA AAC ACA	GAA TAT AAT
1467	1476	1485	1494	1503	1512
AAA ATG AGA	TAA TCT AGC	TTA AAA CTA	TAA CTT CCT	CTT CAG AAC	TCC CAA CCA
1521	1530	1539	1548	1557	1566
CAT TGG ATC	TCA GAA AAA	TGC TGT CTT	CAA AAT GAC	TTC TAC AGA	GAA GAA ATA
1575	1584	1593	1602	1611	1620
ATT TTT CCT	CTG GAC ACT	AGC ACT TAA	GGG GAA GAT	TGG AAG TAA	AGC CTT GAA
1629	1638	1647	1656	1665	1674
AAG AGT ACA	TTT ACC TAC	GTT AAT GAA	AGT TGA CAC	ACT GTT CTG	AGA GTT TTC
1683	1692	1701	1710	1719	1728
ACA GCA TAT	GGA CCC TGT	TTT TCC TAT	TTA ATT TTC	TTA TCA ACC	CTT TAA TTA
1737	1746	1755	1764	1773	1782
GGC AAA GAT	ATT ATT AGT	ACC CTC ATT	GTA GCC ATG	GGA AAA TTG	ATG TTC AGT
1791	1800	1809	1818	1827	1836
GGG GAT CAG	TGA ATT AAA	TGG GGT CAT	ACA AGT ATA	AAA ATT AAA	AAA AAA AAA
1845	1854	1863	1872	1881	1890
GAC TTC ATG	CCC AAT CTC	ATA TGA TGT	GGA AGA ACT	GTT AGA GAG	ACC AAC AGG
1899	1908	1917	1926	1935	1944
GTA GTG GGT	TAG AGA TTT	CCA GAG TCT	TAC ATT TTC	TAG AGG AGG	TAT TTA ATT
1953	1962	1971	1980	1989	1998
TCT TCT CAC	TCA TCC AGT	GTT GTA TTT	AGG AAT TTC	CTG GCA ACA	GAA CTC ATG
2007	2016	2025	2034	2043	2052
GCT TTA ATC	CCA CTA GCT	ATT GCT TAT	TGT CCT GGT	CCA ATT GCC	AAT TAC CTG
2061	2070	2079	2088	2097	2106
TGT CTT GGA	AGA AGT GAT	TTC TAG GTT	CAC CAT TAT	GGA AGA TTC	TTA TTC AGA
2115	2124	2133	2142	2151	2160
AAG TCT GCA	TAG GGC TTA	TAG CAA GTT	ATT TAT TTT	TAA AAG TTC	CAT AGG TGA
2169	2178	2187	2196	2205	2214
TTC TGA TAG	GCA GTG AGG	TTA GGG AGC	CAC CAG TTA	TGA TGG GAA	GTA TGG AAT
2223	2232	2241	2250	2259	2268
GGC AGG TCT	TGA AGA TAA	CAT TGG CCT	TTT GAG TGT	GAC TCG TAG	CTG GAA AGT
2277	2286	2295	2304	2313	2322
GAG GGA ATC	TTC AGG ACC	ATG CTT TAT	TTG GGG CTT	TGT GCA GTA	TGG AAC AGG
2331	2340	2349	2358	2367	2376
GAC TTT GAG	ACC AGG AAA	GCA ATC TGA	CTT AGG CAT	GGG AAT CAG	GCA TTT TTG

FIG. 2D

2385	2394	2403	2412	2421	2430
CTT CTG AGG GGC TAT TAC CAA GGG TTA ATA GGT TTC ATC TTC AAC AGG ATA TGA					
2439	2448	2457	2466	2475	2484
CAA CAG TGT TAA CCA AGA AAC TCA AAT TAC AAA TAC TAA AAC ATG TGA TCA TAT					
2493	2502	2511	2520	2529	2538
ATG TGG TAA GTT TCA TTT TCT TTT TCA ATC CTC AGG TTC CCT GAT ATG GAT TCC					
2547	2556	2565	2574	2583	2592
TAT AAC ATG CTT TCA TCC CCT TTT GTA ATG GAT ATC ATA TTT GGA AAT GCC TAT					
2601	2610	2619	2628	2637	2646
TTA ATA CTT GTA TTT GCT GCT GGA CTG TAA GCC CAT GAG GGC ACT GTT TAT TAT					
2655	2664	2673	2682	2691	2700
TGA ATG TCA TCT CTG TTC ATC ATT GAC TGC TCT TTG CTC ATC ATT GAA TCC CCC					
2709	2718	2727	2736	2745	2754
AGC AAA GTG CCT AGA ACA TAA TAG TGC TTA TGC TTG ACA CCG GTT ATT TTT CAT					
2763	2772	2781	2790	2799	2808
CAA ACC TGA TTC CTT CTG TCC TGA ACA CAT AGC CAG GCA ATT TTC CAG CCT TCT					
2817	2826	2835	2844	2853	2862
TTG AGT TGG GTA TTA TTA AAT TCT GGC CAT TAC TTC CAA TGT GAG TGG AAG TGA					
2871	2880	2889	2898	2907	2916
CAT GTG CAA TTT CTA TAC CTG GCT CAT AAA ACC CTC CCA TGT GCA GCC TTT CAT					
2925	2934	2943	2952	2961	2970
GTT GAC ATT AAA TGT GAC TTG GGA AGC TAT GTG TTA CAC AGA GTA AAT CAC CAG					
2979	2988	2997	3006	3015	3024
AAG CCT GGA TTT CTG AAA AAA CTG TGC AGA GCC AAA CCT CTG TCA TTT GCA ACT					
3033	3042	3051	3060	3069	3078
CCC ACT TGT ATT TGT ACG AGG CAG TTG GAT AAG TGA AAA ATA AAG TAC TAT TGT					
3087	3096	3105	3114	3123	3132
GTC AAG AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA					

AAA A 3'

Figure 3: Protein Sequence for 101P3A11.

MVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYTVRTEHSLHEPMYIFLCMLSGIDILI
STSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGV
AAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDDIRVNVVYGLIVIISAIGLDSLLISFSYL
LILKTVLGLTREAQAKAFGTCVSHVCAVFIFYVFFIGLSMVHRFSKRRDSPLPVILANTYLLVPPVLNPIVYG
VKTKEIRQRILRLFHVATHASEP

Figure 4

Alignment of 101P3A11 (Sbjct) with mouse olfactory receptor S25 (Query)

Query: 34 GNYTVVTEFILLGLTDDITVSVILFVMFLIVYSVTLMGNLNIIVLIRTSPQLHTPMYLFL 93
 GN + T FIL+GL L +Y + ++GNL II ++RT LH PMY+FL

Sbjct: 6 GNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFL 65

Query: 94 SHLAFLDIGYSSSVTPIMLRGFLRKGTFFIPVAGCVAQLCIVVAFGTSESFLLASMAYDRY 153
 L+ +DI S+S P ML F T I C+ Q+ + + ES +L +MA+DRY

Sbjct: 66 CMLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRY 125

Query: 154 VAICSPLLYSTQMSSTVCILLVGTSYLGWVNAWIFTGCSLNLSCGPNKINHFFCDYSP 213
 VAIC PL ++T ++ + + + G L FC N ++H +C +

Sbjct: 126 VAICHPLRHATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQD 185

Query: 214 LLKLSCSHDFSFEVIPAISGSIIVVTVFIIALSYVYILVSILKMRSTEGRQKAFSTCTS 273
 ++KL+C V I S I + +I+ SY+ IL ++L + + E + KAF TC S

Sbjct: 186 VMKLACDDIRNVVYGLLIVIIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVS 244

Query: 274 HLTAVTLFFGTITFIYVMPQSSYSTDQNK----VVSVFYTVVIPMLNPLIYSFRNKEVKE 329
 H+ AV +F+ + FI + +S ++ +++ Y +V P+LNP++Y + KE+++

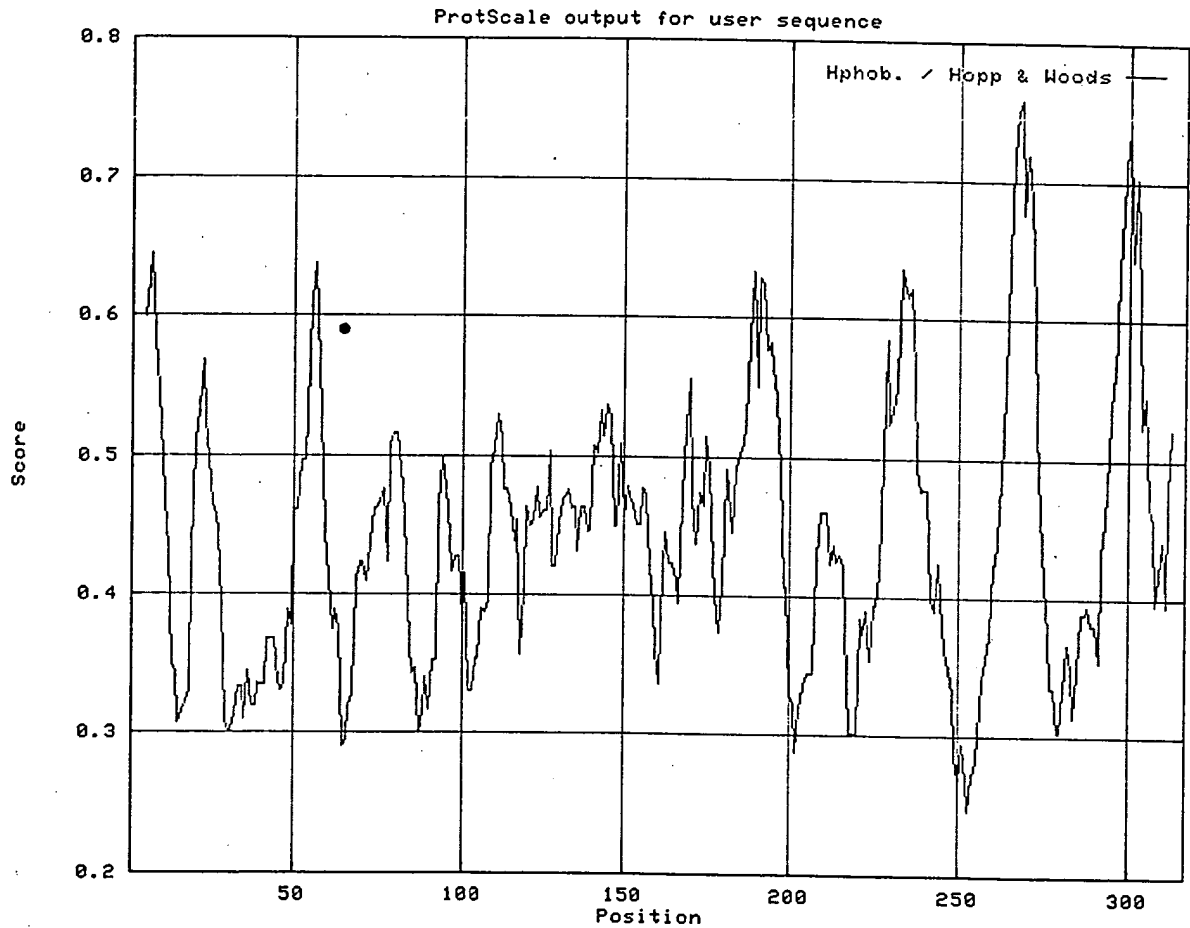
Sbjct: 245 HVCAVFIFY--VPFIGLSMVHRFSKRDRSPLPVILANIYLLVPPVLNPIVYGVKTKEIRQ 302

Query: 330 AMKKL 334
 + +L

Sbjct: 303 RILRL 307



Figure 5:
101P3A11 Hydrophilicity profile
(Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828)



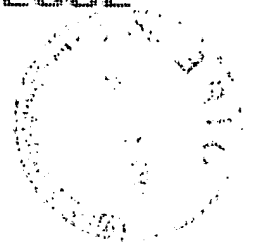


Figure 6:
101P3A11 Hydropathicity Profile
(Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132)

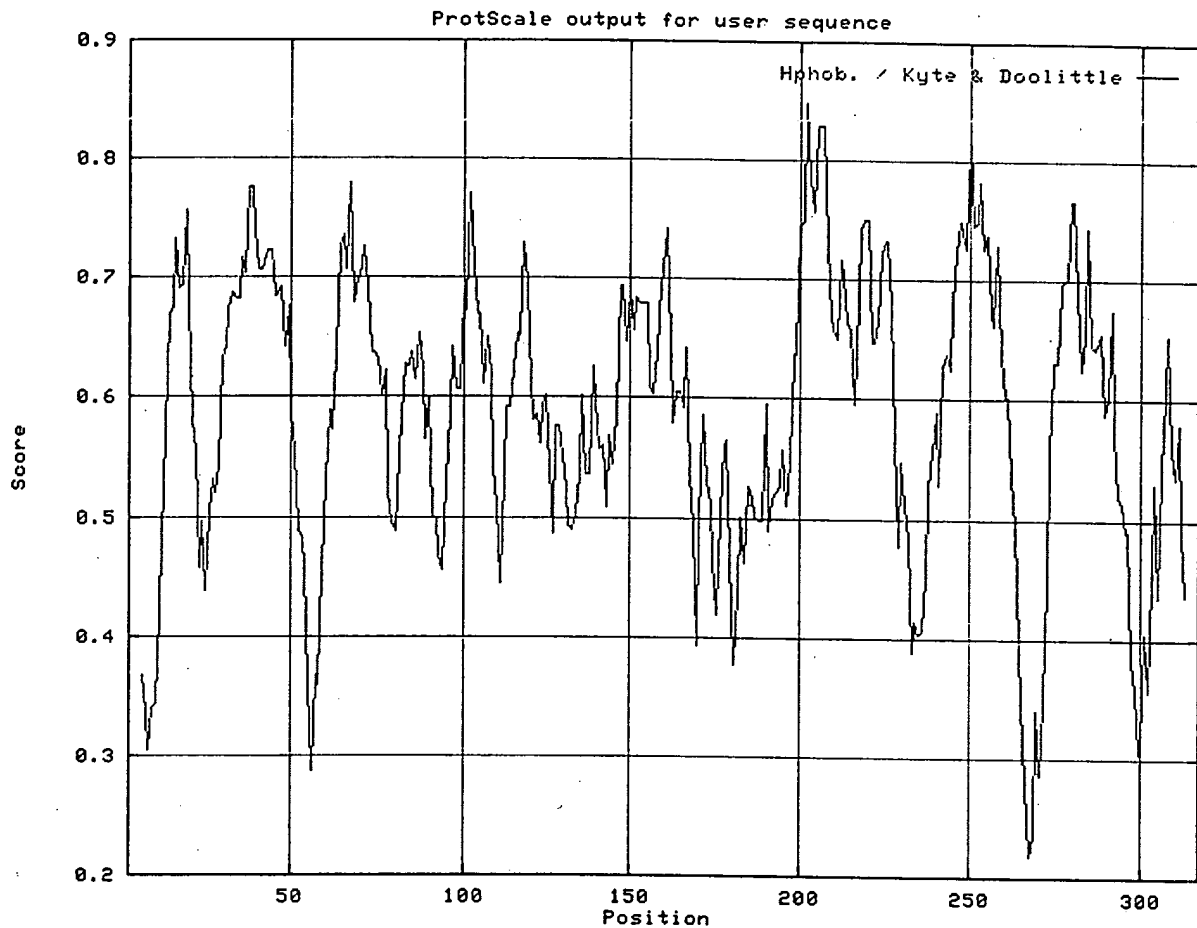




Figure 7:
101P3A11 % Accessible Residues Profile
(Janin J., 1979. Nature 277:491-492)

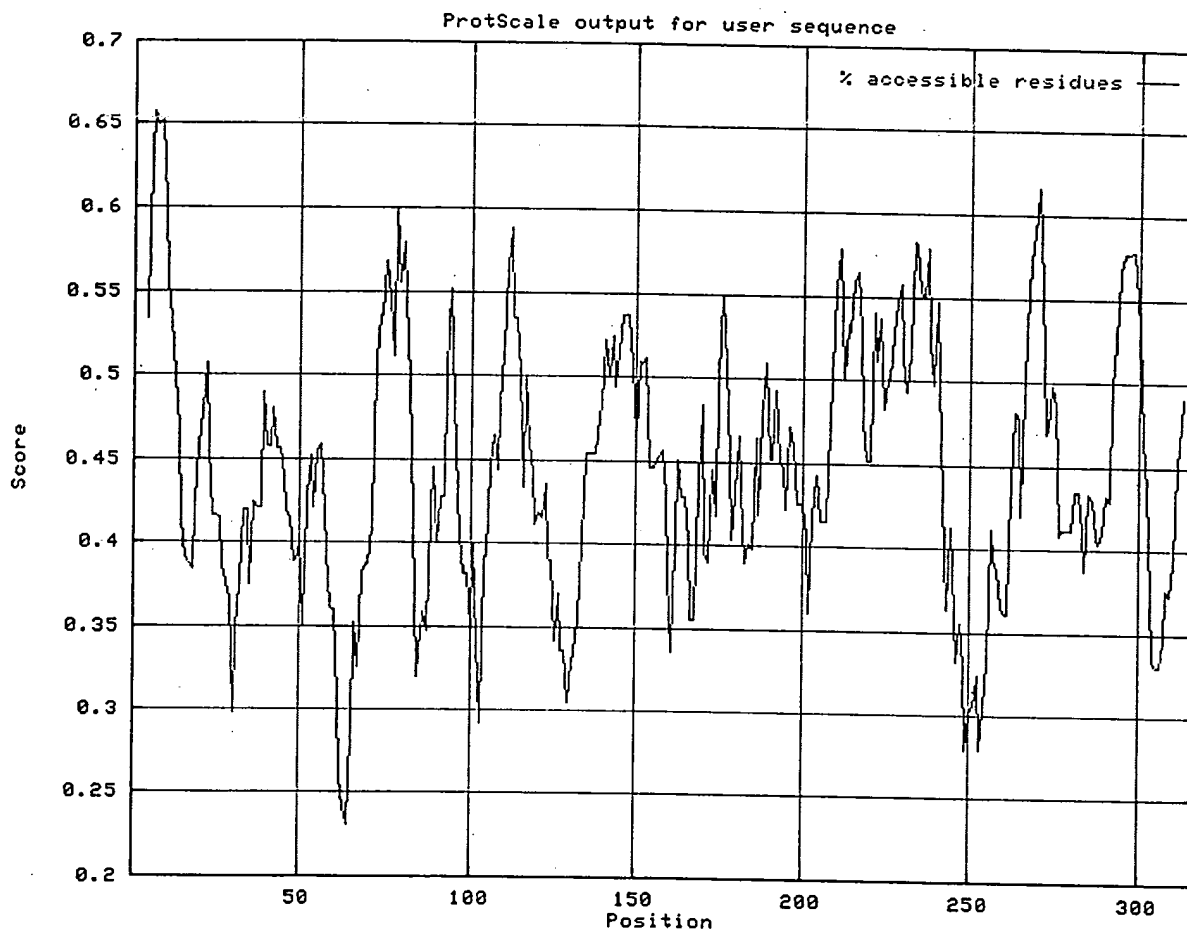




Figure 8:
101P3A11 Average Flexibility Profile
(Bhaskaran R., Ponnuswamy P.K., 1988.
Int. J. Pept. Protein Res. 32:242-255)

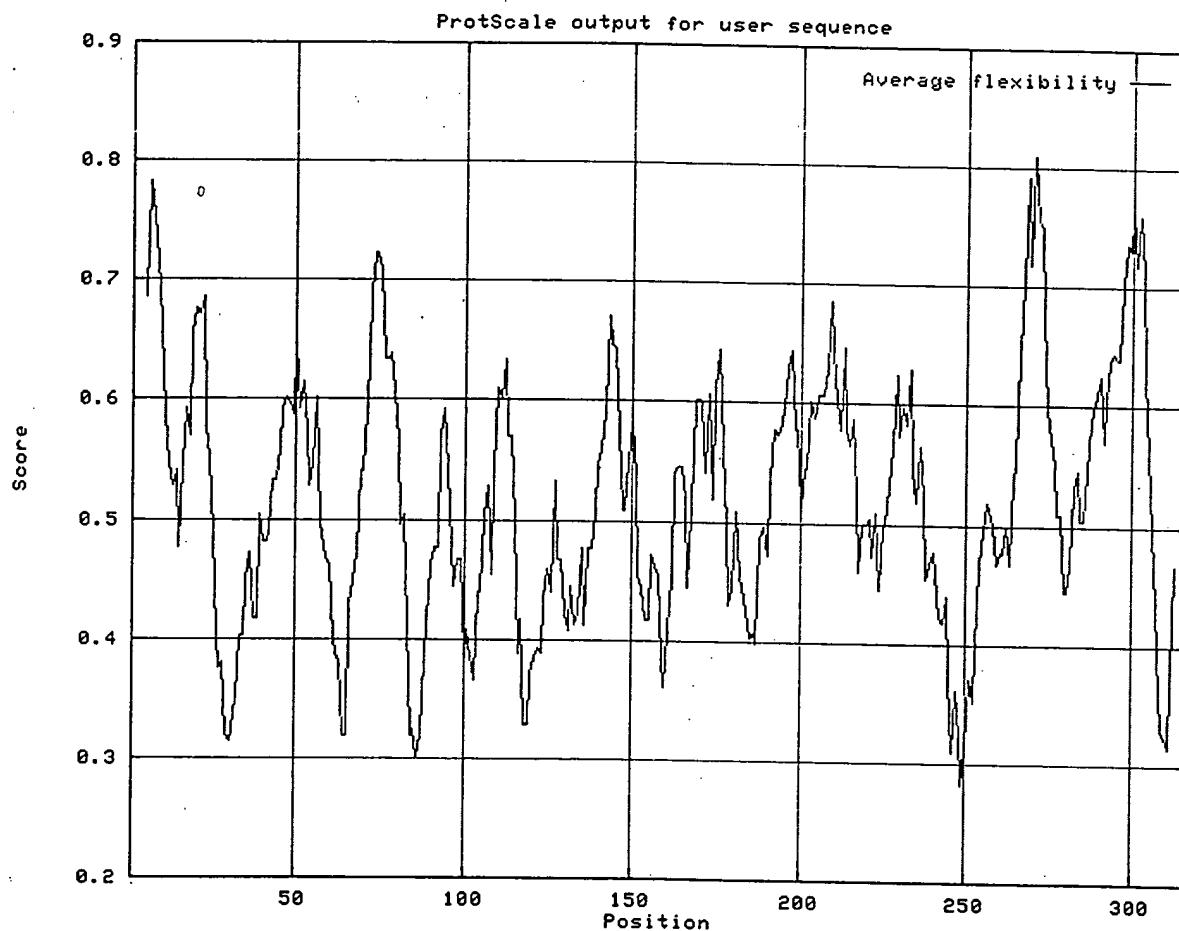




Figure 9:
101P3A11 Beta-turn Profile
(Deleage, G., Roux B. 1987. Protein Engineering 1:289-294)

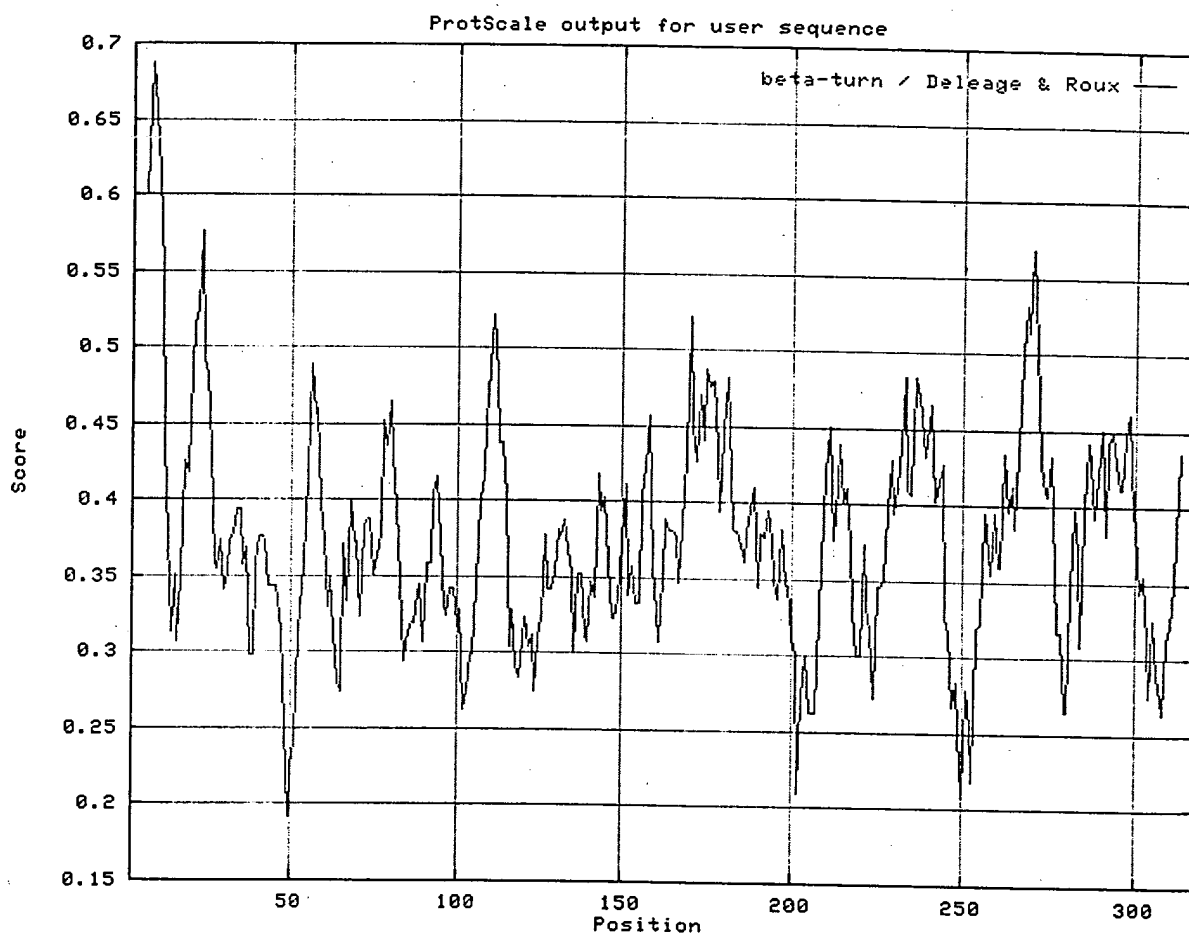
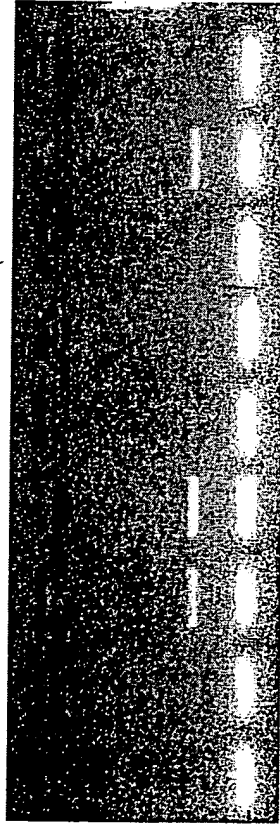


Figure 10A. Expression of 101P3A11 by RT-PCR

1 2 3 4 5 6 7 8 9



- VP1 (Kidney, Lung, Liver)
- VP2 (Pancreas, Colon, Stomach)
- Prostate xenograft Pool
- Prostate Cancer Pool
- Kidney Cancer Pool
- Colon Cancer Pool
- Breast Cancer Pool
- Metastasis Pool
- H2O





Figure 10B

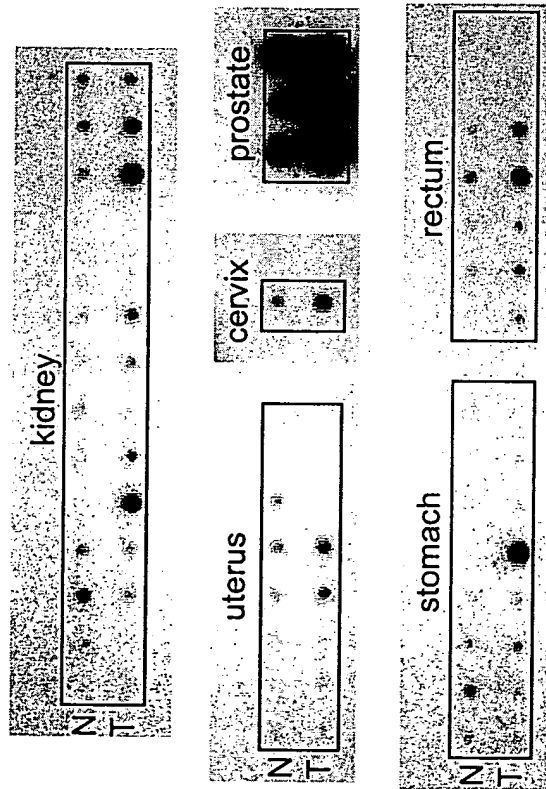
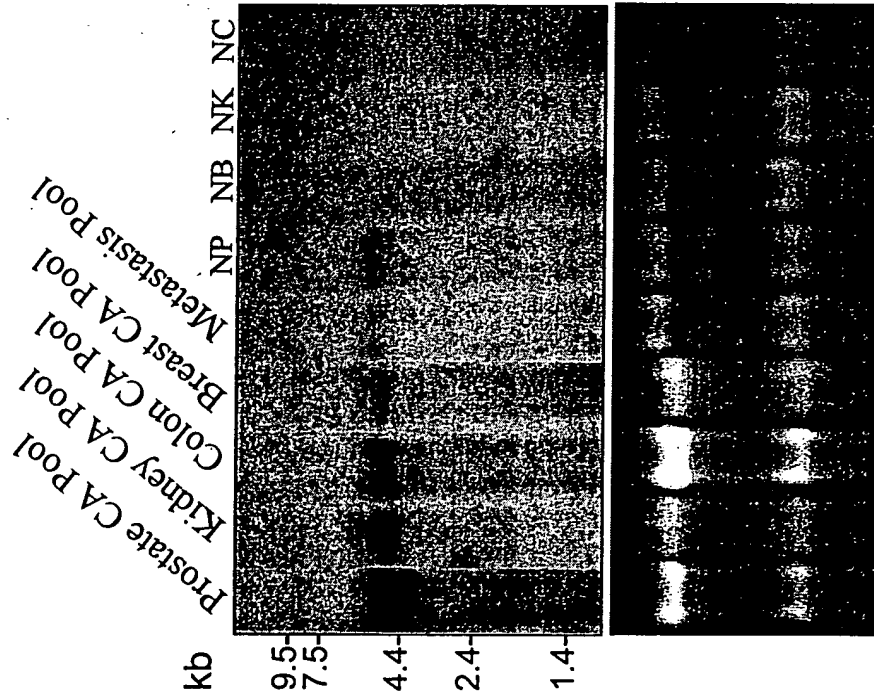


Figure 11. Expression of 101P3A11 in Human Patient Cancer Specimens



10µg total RNA/per lane from a pool of 3 tumors as follows:

Prostate Cancer Pool = gleason 6, 8, 9

Kidney Cancer Pool = grade 2, 2, 3

Colon Cancer Pool = stage II, III, IV

Breast Cancer Pool = grade 1, 2, 3

Metastasis Pool = colon to lung, colon to liver, ovary to fall. tube

NP = Normal Prostate

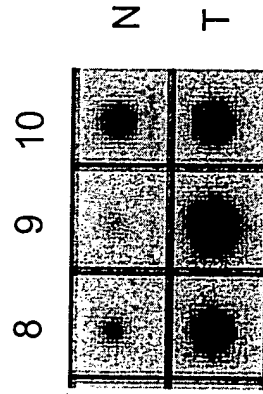
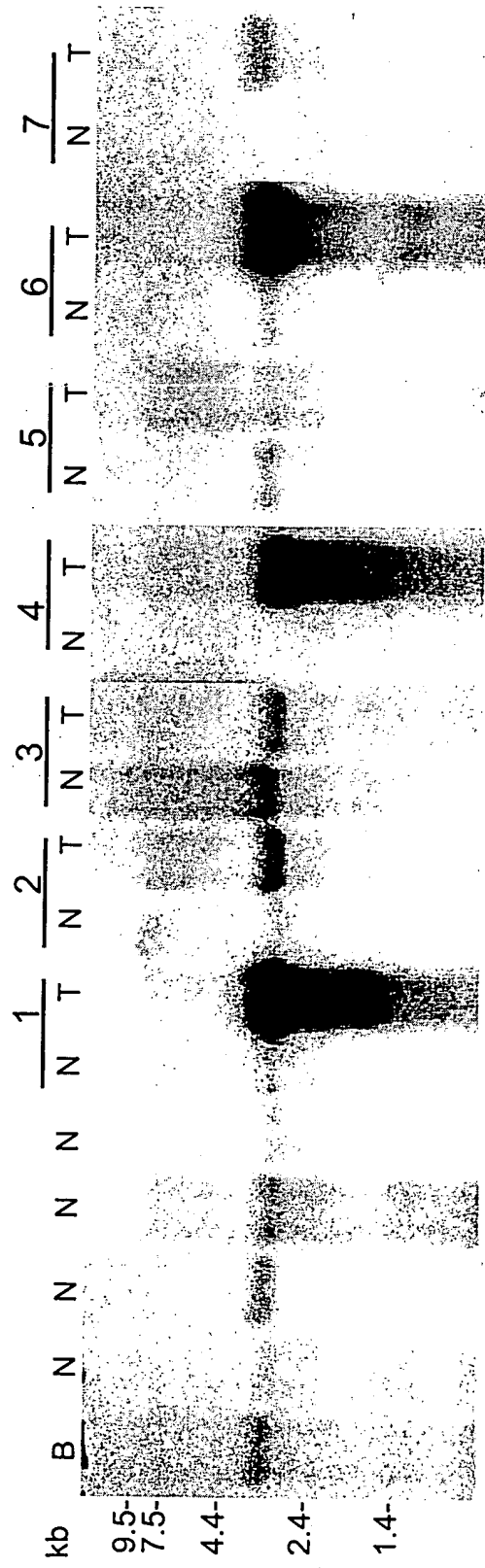
NB = Normal Bladder

NK = Normal Kidney

NC = Normal Colon



Figure 12A



Matched tumor/normal patient samples:

B = BPH
N = Normal
T = Tumor



Figure 12B and 12C

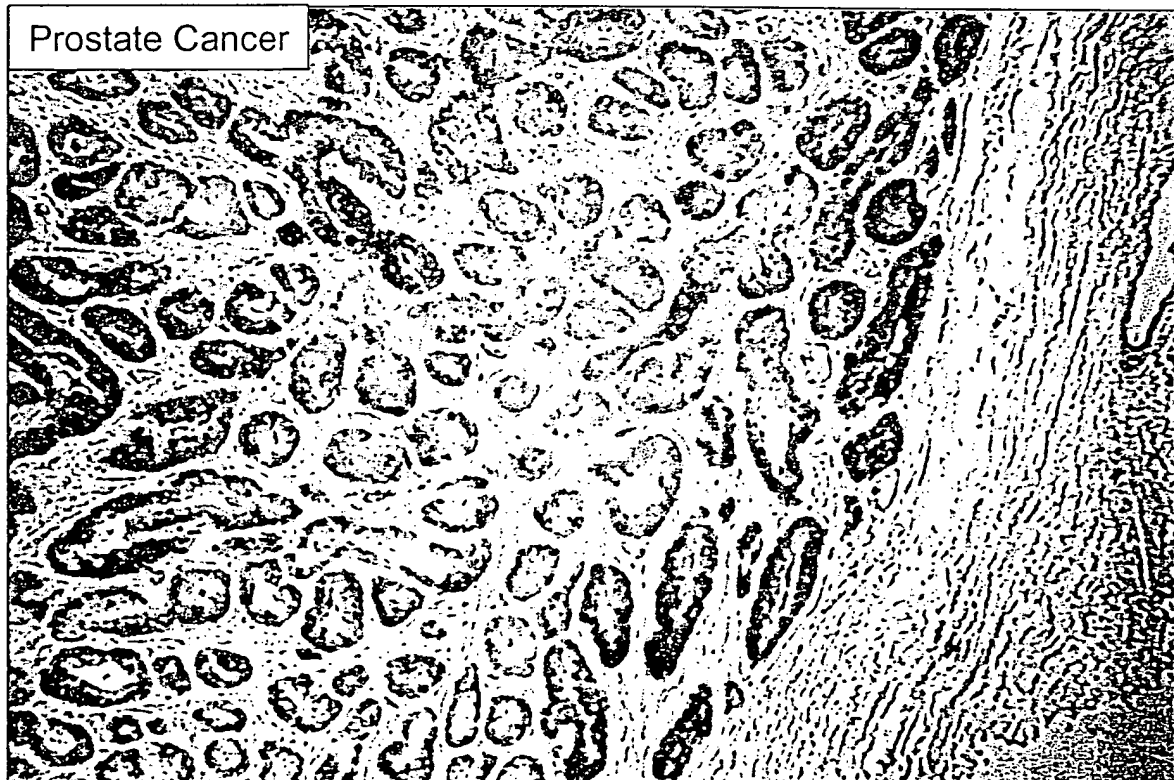


Figure 12D and 12E

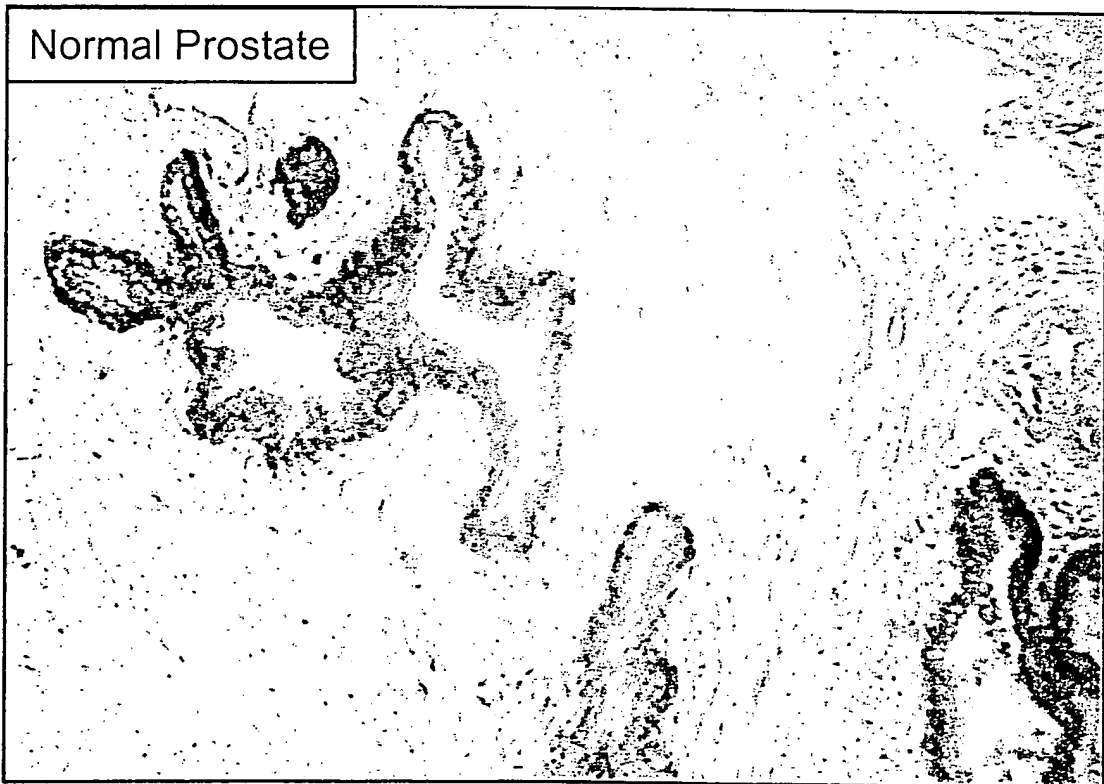
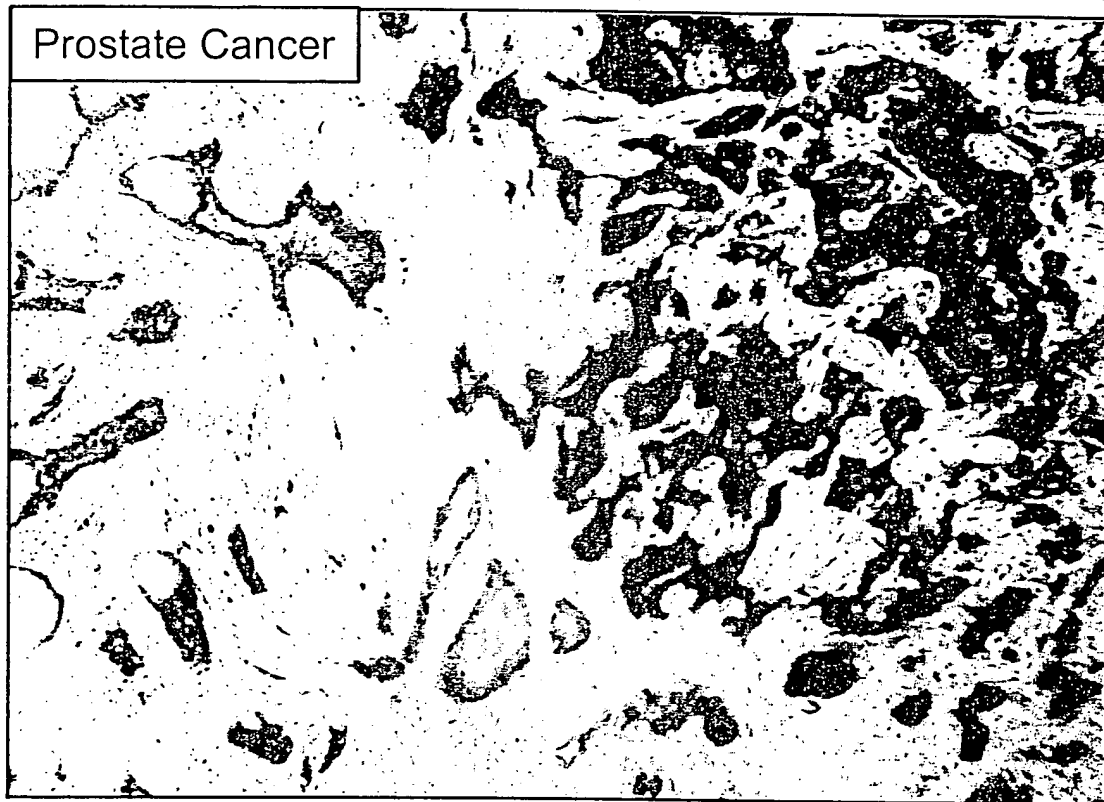
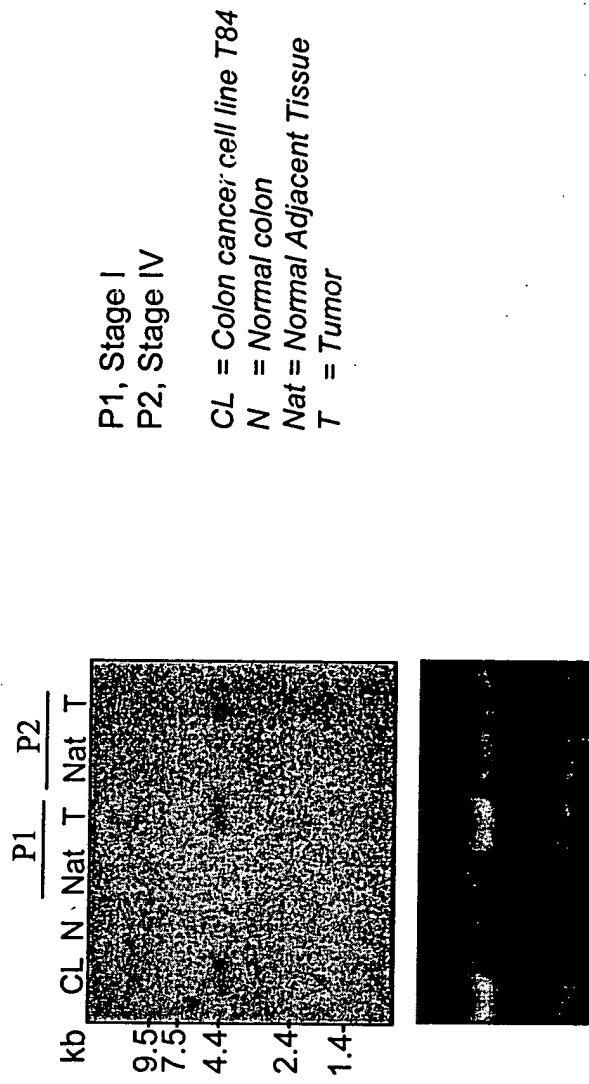


Figure 13. Expression of 101P3A11 in Colon Cancer Patient Specimens

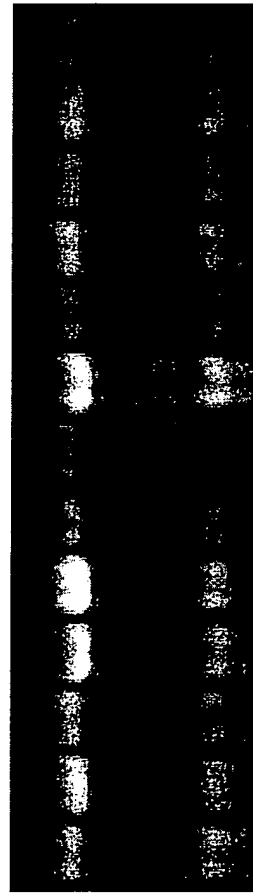
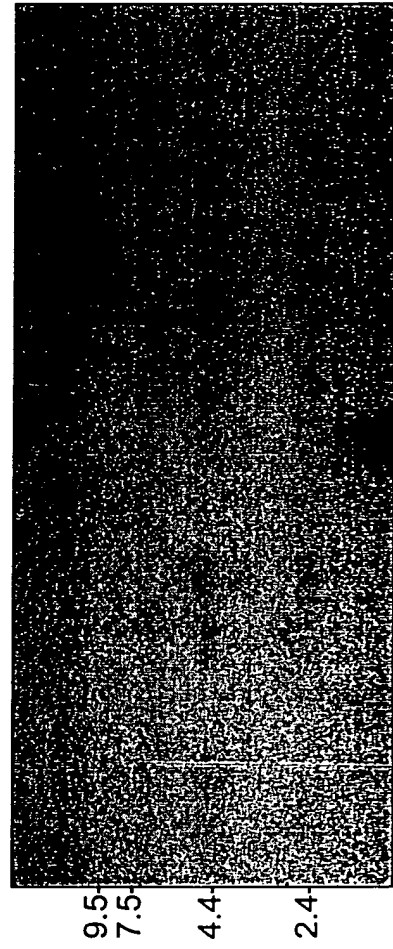


10001469 .052002



Figure 14. Expression of 101P3A11 in Kidney Cancer Patient Specimens

Pt.1		Pt.2		Pt.3		Pt.4		Pt.5		Pt.6	
N	T	N	T	N	T	N	T	N	T	N	T



Pt.1, Papillary carcinoma, stage I
 Pt.2, Invasive papillary carcinoma
 Pt.3, Clear cell type grade 1/3, focally 2/3
 Pt.4, Clear cell type, stage III
 Pt.5, Clear cell type, stage III
 Pt.6, Clear cell type, stage III

N = Normal kidney
 NAT = Normal adjacent tumor
 T = Tumor



Figure 15A-15C. Androgen Regulation of 101P3A11 in Tissue Culture Cells

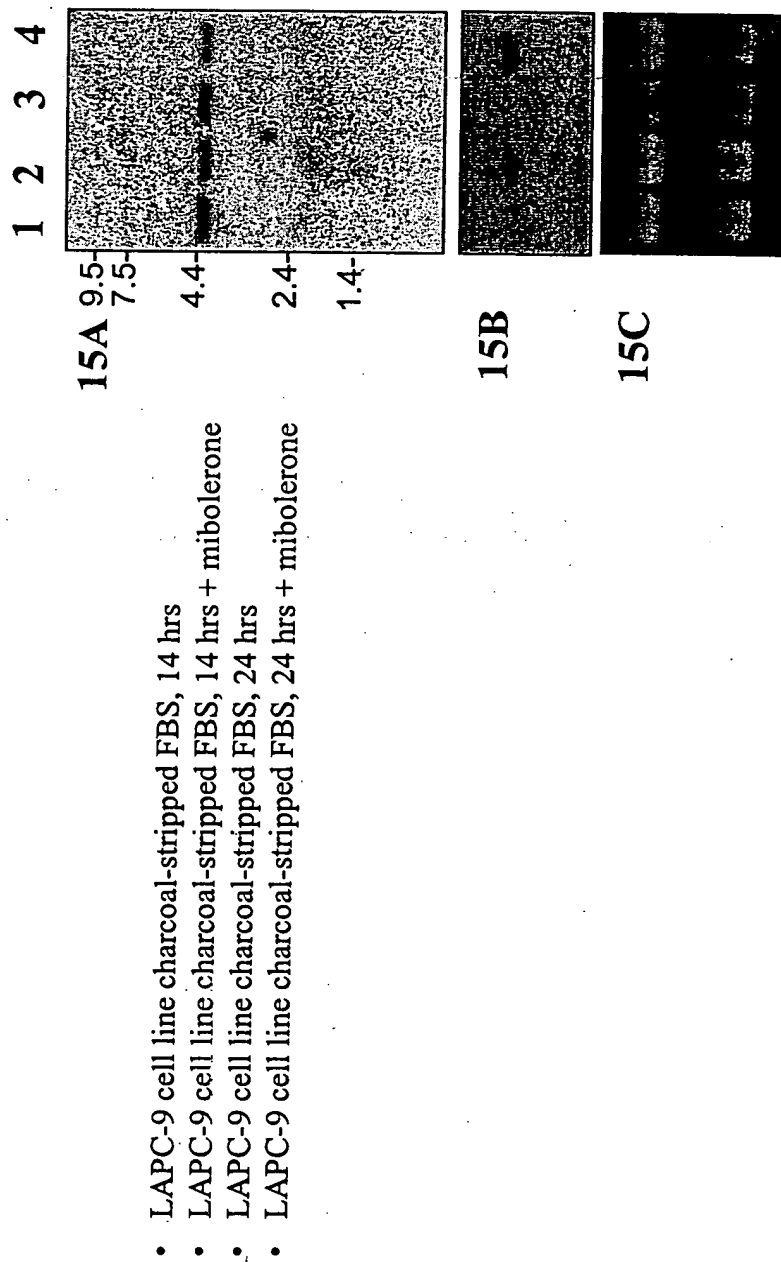


Figure 16. Androgen Regulation of 101P3A11 *In Vivo*

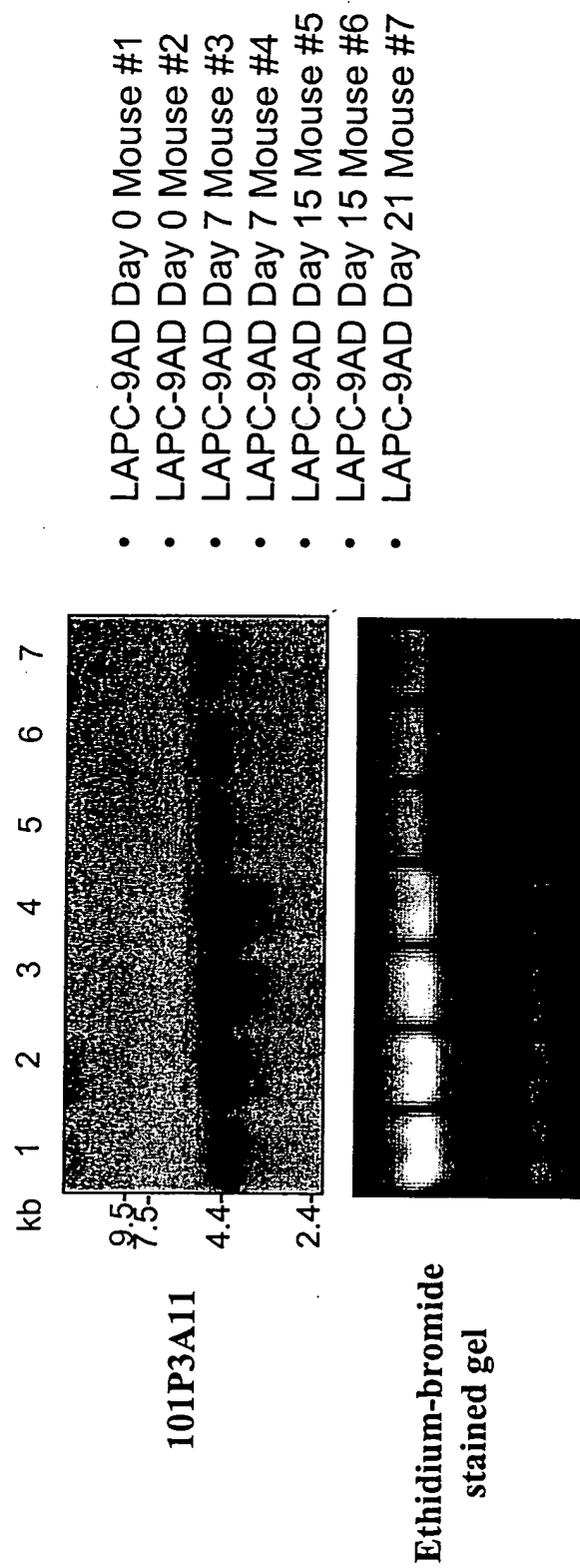


Figure 17. Expression and Detection of 101P3A11(159-202)-psecFc Fusion Protein

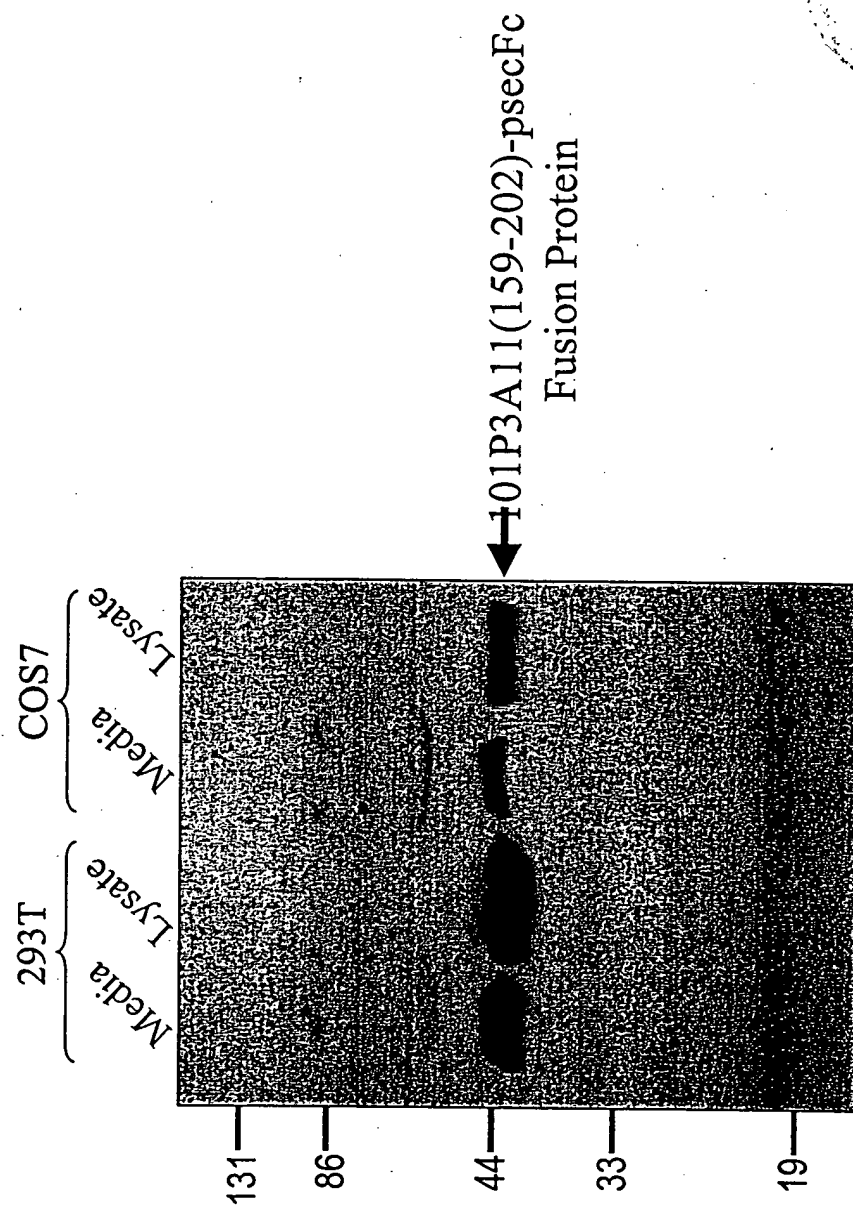


Figure 18. Expression of 101P3A11 in 300.19 Cells

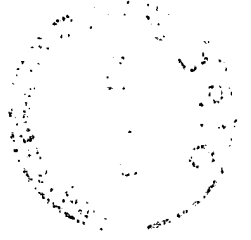
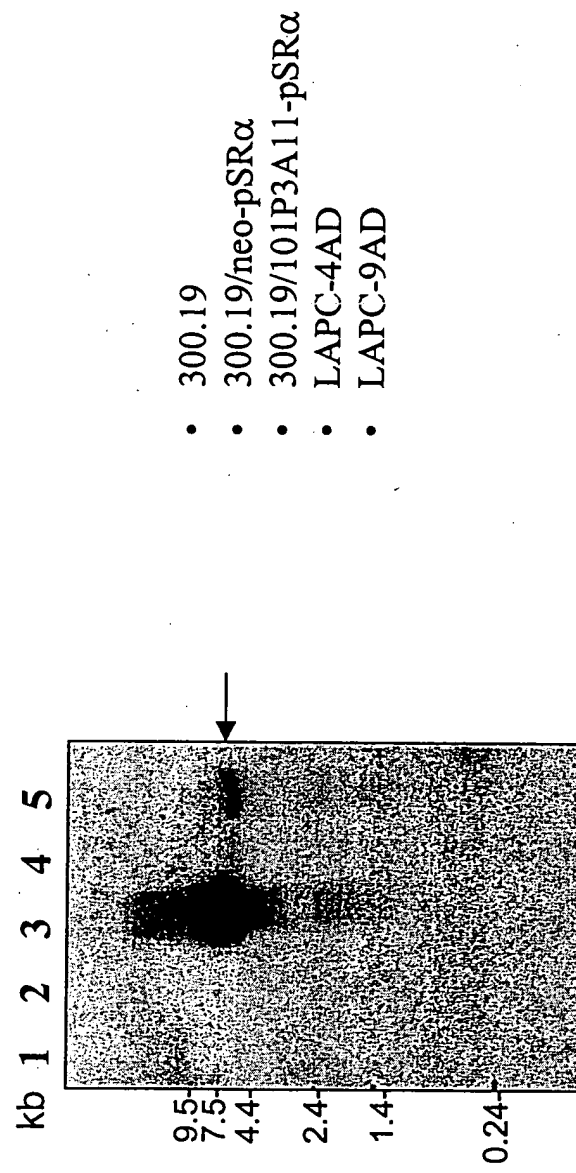
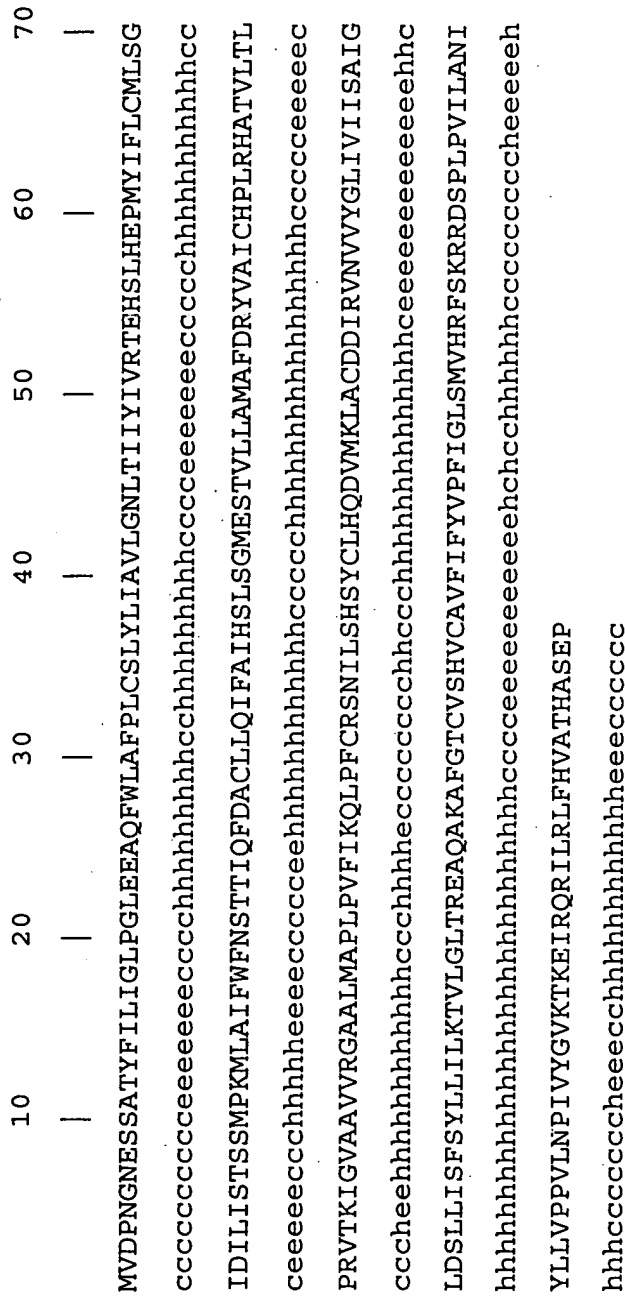


Figure 19A. Secondary structure prediction of 101P3A11



c: random coil (30.60%)
e: extended strand (21.45%)
h: alpha helix (47.95%)

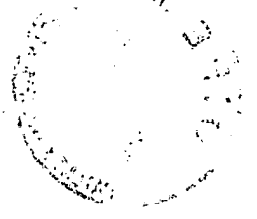
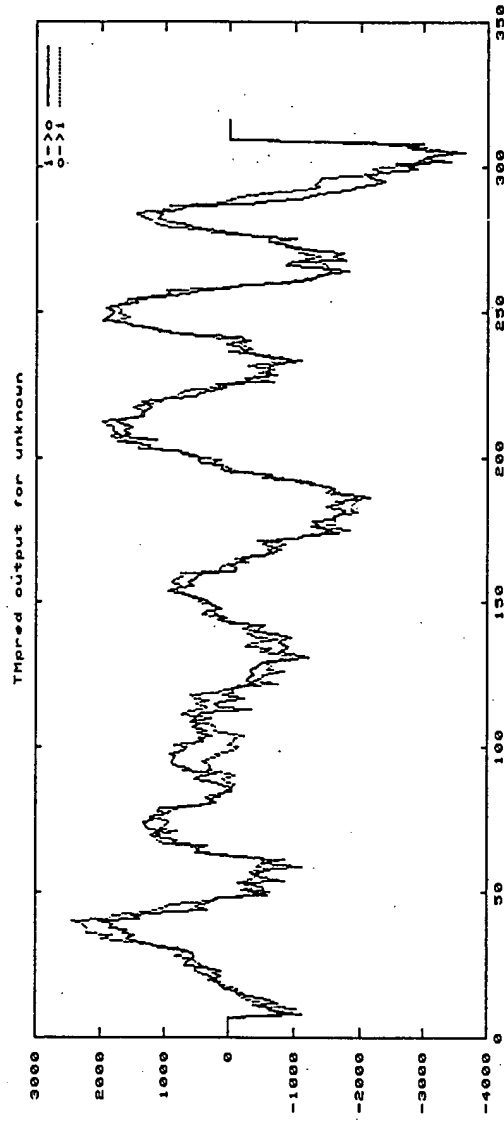
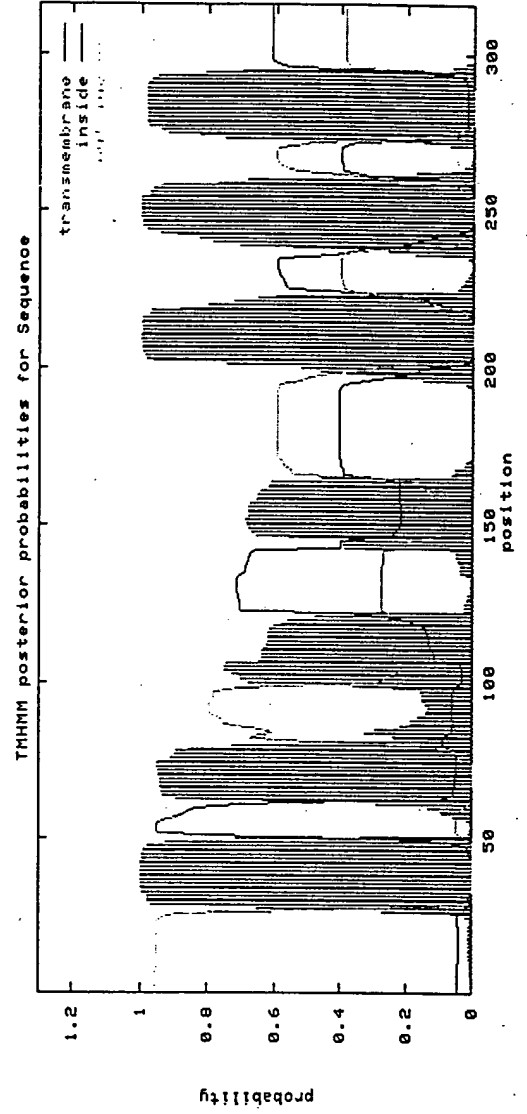


Figure 19B-19C. Transmembrane prediction of 101P3A11

19B



19C



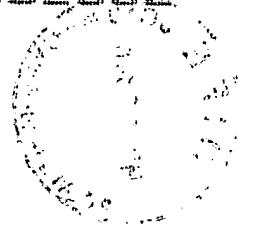


Figure 20. Expression of 101P3A11 in NIH-3T3 Tumors

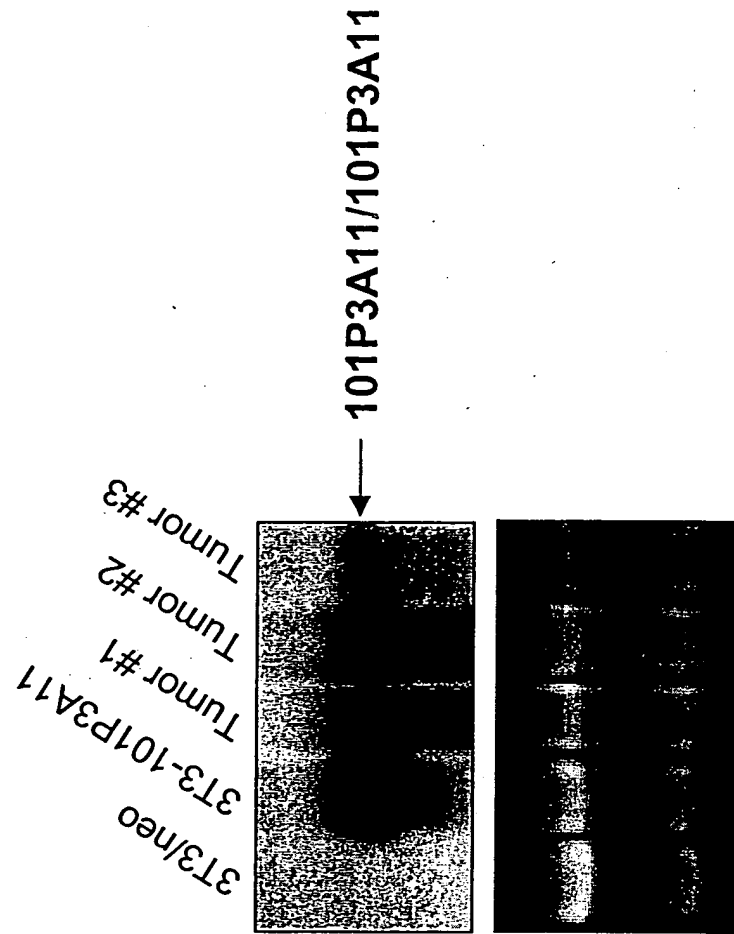
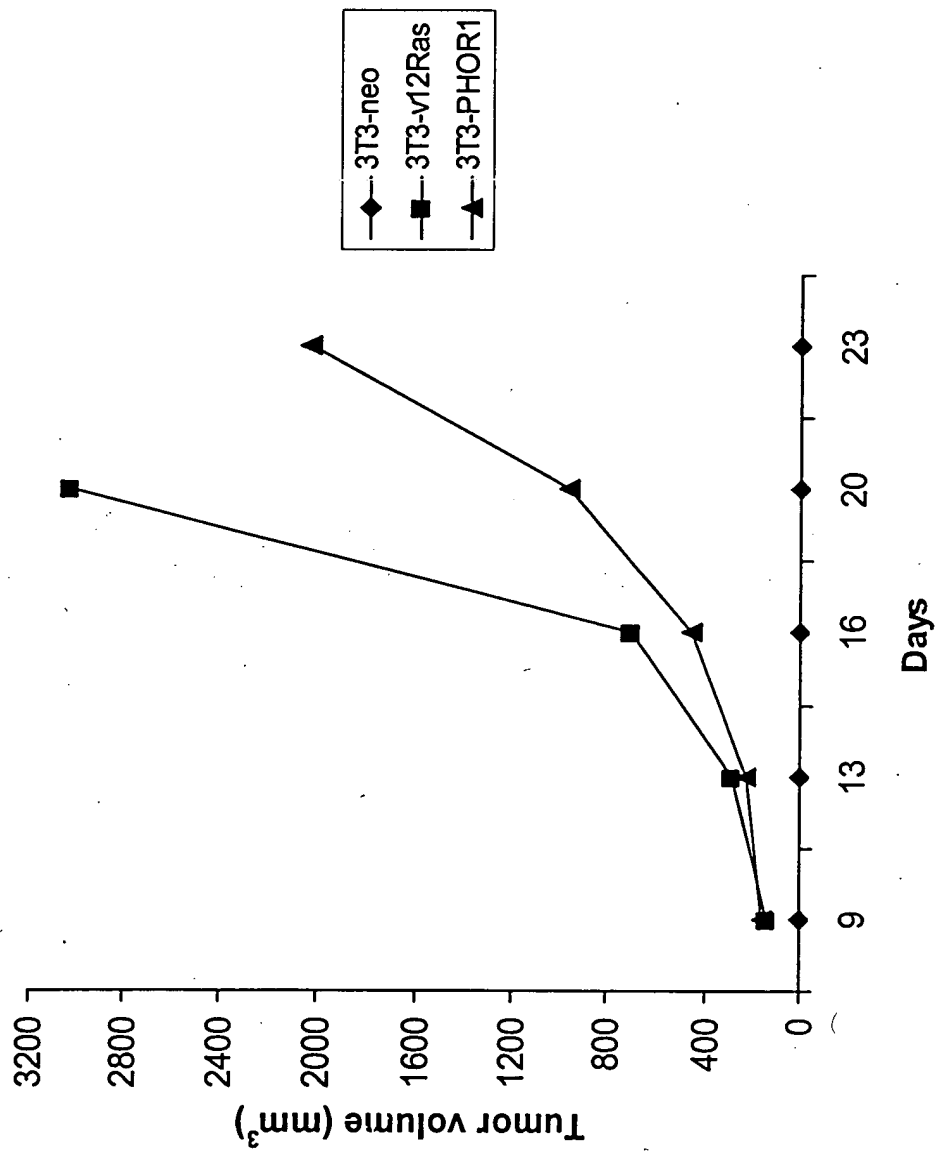
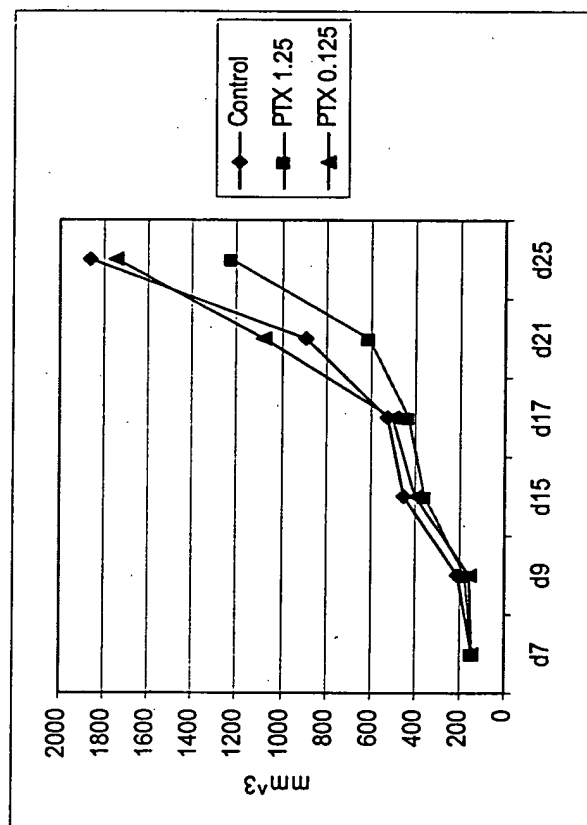


Figure 21: 101P3A11 Induces Tumor Formation of 3T3 Cells



• Injection of 10^6 3T3-neo, 3T3-Ras or 3T3-101P3A11 cells subcutaneously into SCID mice revealed that 6/6 3T3-Ras-injected mice formed tumors, 6/6 3T3-101P3A11-injected mice formed tumors, and 0/6 3T3-neo-injected mice formed tumors.

Figure 22: PTX Reduces the *in vivo* Growth of 3T3-101P3A11 Tumors



- Pertussis toxin inhibits the sub-cutaneous growth of 3T3-101P3A11 tumors in SCID mice.
- The inhibitory activity of pertussis toxin occurs in a dose dependent manner.



Figure 23: Alignment of 101P3A11-PHOR-1 with the rat GPCR RA1C (gi|3420759).

Identities = 179/299 (59%), Positives = 231/299 (76%), Gaps = 1/299 (0%)

```

PHOR: 14  FILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73
        F+LIG+PGLEEA FW  FPL S+Y +A+ GN  +++IVRTE SLH PMY+FLCML+ ID+
RA1C: 11  FMLIGIPGLEEAHFWFGFPLLSMYAVALFGNCIVVFIVRTERS LHAPMYLFLCMLAIDL 70

PHOR: 74  LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLR 133
        +STS+MPK+LA+FWF+S  I FDACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR
RA1C: 71  ALSTSTMPKILALFWFDSREITFDACLAQMFFIHLSAIESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDD 193
        HA VL      +IG+ A+VRG+      PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D
RA1C: 131 HAAVLNNTVTQIGMVALVRGSLFFFPLPLLIKRLAFCHSNVLSHSYCVHQDVMKLAYTD 190

PHOR: 194 IRVNVVYGLLIVIIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCAVFIF 252
        NVVYGL  I+  +G+D + IS SY LI++ VL L ++  +AKAFGTCVSH+  V F
RA1C: 191 TLPNVVYGLTAILLVMGVDVMFISLSYFLIIRAVLQLPSKSERAKAFGTCVSHIGVVLA 250

PHOR: 253 YVPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLNPIVYGVKTKAIRQIRILRLFHVA 311
        YVP IGLS+VHRF      D  + V++ ++YLL+PPV+NPI+YG KTK+IR R+L +F ++
RA1C: 251 YVPLIGLSVVRFGNSLDPIVHVLMGDVYLLPPVINPIIYGAKTKQIRTRVLAMFKIS 309

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Figure 24: Alignment of 101P3A11-PHOR-1 with the human prostate specific GPCR. (gi|13540539)

Identities = 179/299 (59%), Positives = 233/299 (77%), Gaps = 1/299 (0%)

PHOR: 14 FILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73
F+LIG+PGLE+A FW+ FPL S+Y++A+ GN +++IVRTE SLH PMY+FLCML+ ID+

GPCR: 11 FVLIGIPGLEKAHFWVGFPLLSMYVVMFGNCIVVFIVRTERS LHAPMYLFLCMLAIDL 70

PHOR: 74 LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLR 133
+STS+MPK+LA+FWF+S I F+ACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR

GPCR: 71 ALSTSTMPKILALFWFDSREISFEACLTQMFFIHLSAIESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDD 193
HA VL +IG+ AVVRG+ PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D

GPCR: 131 HAAVLNNTVTAQIGIVAVVRGSLFFFPLPLLIKRLAFCHSNVLSHSYCVHQDVMKLAYAD 190

PHOR: 194 IRVNVVYGLIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCAVFIF 252
NVVYGL I+ +G+D + IS SY LI++TVL L ++ +AKAFGTCVSH+ V F

GPCR: 191 TLPNVVYGLTAILLVMGVDVMFISLSYFLIIRTVLQLPSKSERAKAFGTCVSHIGVVLAF 250

PHOR: 253 YVPFIGLSMVHRFSKRDRSPLPVILANIYLLVPPVLNPIVYGVKTKAIRQLRLFHVA 311
YVP IGLS+VHRF + V++ +IYLL+PPV+NPI+YG KTK+IR R+L +F ++

GPCR: 251 YVPLIGLSVVHRFGNSLHPIVRVVMGDIYLLLPVINPIIYGAKTKQIRTRVLAMFKIS 309

Figure 25: Alignment with human olfactory receptor 5II12 (gi|14423836)

Identities = 163/304 (53%), Positives = 214/304 (69%), Gaps = 1/304 (0%)

PHOR: 7 NESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLC 66
 N + +F+L G+PGLE + WL+ PLC +Y +A+ GN I+ VR E SLHEPMY FL
 HOR5: 5 NVTHPAFFLLTGIPGLESSHWSLGPCLVMYAVALGGNTVILQAVRVEPSLHEPMYIFLS 64

PHOR: 67 MLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYV 126
 MLS D+ IS +++P +L F N+ I FDACL+Q+F IH S MES +LLAM+FDYV
 HOR5: 65 MLSFSDVAISMATLPTVLRFTCLNARNITFDACLIQMFLIHFFSMMESGILLAMSFDYV 124

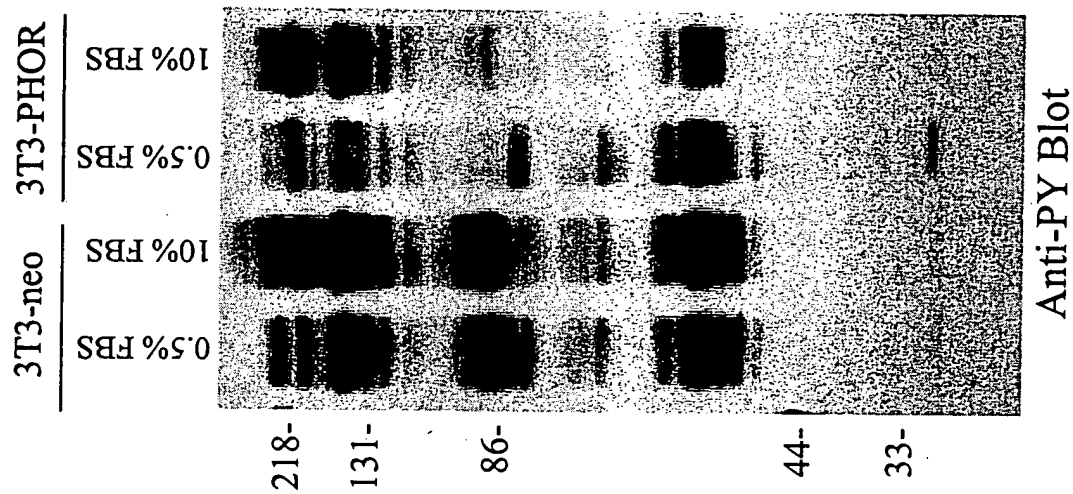
PHOR: 127 AICHPLRHATVLTLPRTVKIGVAAVVRGAALMAPLPVFIKQLPFCRSNLSHSYCLHQDV 186
 AIC PLR+ATVLT + +G+ A R + PLP IK+LP CRSN+LSHSYCLH D+
 HOR5: 125 AICDPLRYATVLTTEVIAAMGLGAAARSFITLFPFLIKRPLCRSNVLSHSYCLHPDM 184

PHOR: 187 MKLACDDIRVNVVYGLIVIIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSH 245
 M+LAC DI +N +YGL V++S G+D I SY+LIL++V+ +RE + KA TCVSH
 HOR5: 185 MRLACADISINSIYGLFVLVSTFGMDLFFIFLSYVLILRSVMATASREERLKALNTCVSH 244

PHOR: 246 VCAVFIFYVPFGLSMVHRFSKRRDSPLPVILANIYLLVPPVLNPIVYGVTKEIRQRIL 305
 + AV FYVP IG+S VHRF K + V+++N+YL VPPVLNP++Y KTKEIR+ I
 HOR5: 245 ILAVLAFYVPMIGVSTVHRFGKHVPCYIHVLMNSNVYLFVPPVLNPLIYSAKTKEIRRAIF 304

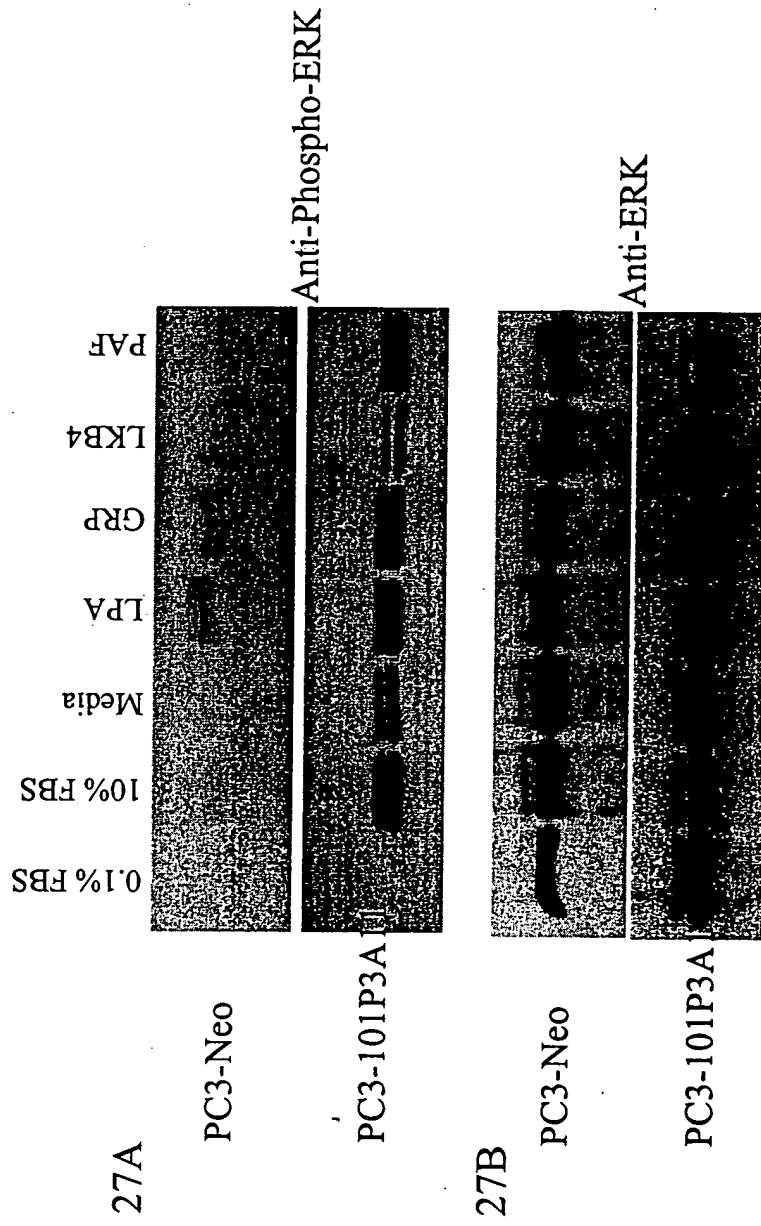
PHOR: 306 RLFH 309
 R+FH
 HOR5: 305 RMFH 308

Figure 26: 101P3A11 Modulated Tyrosine Phosphorylation in NIH-3T3 Cells



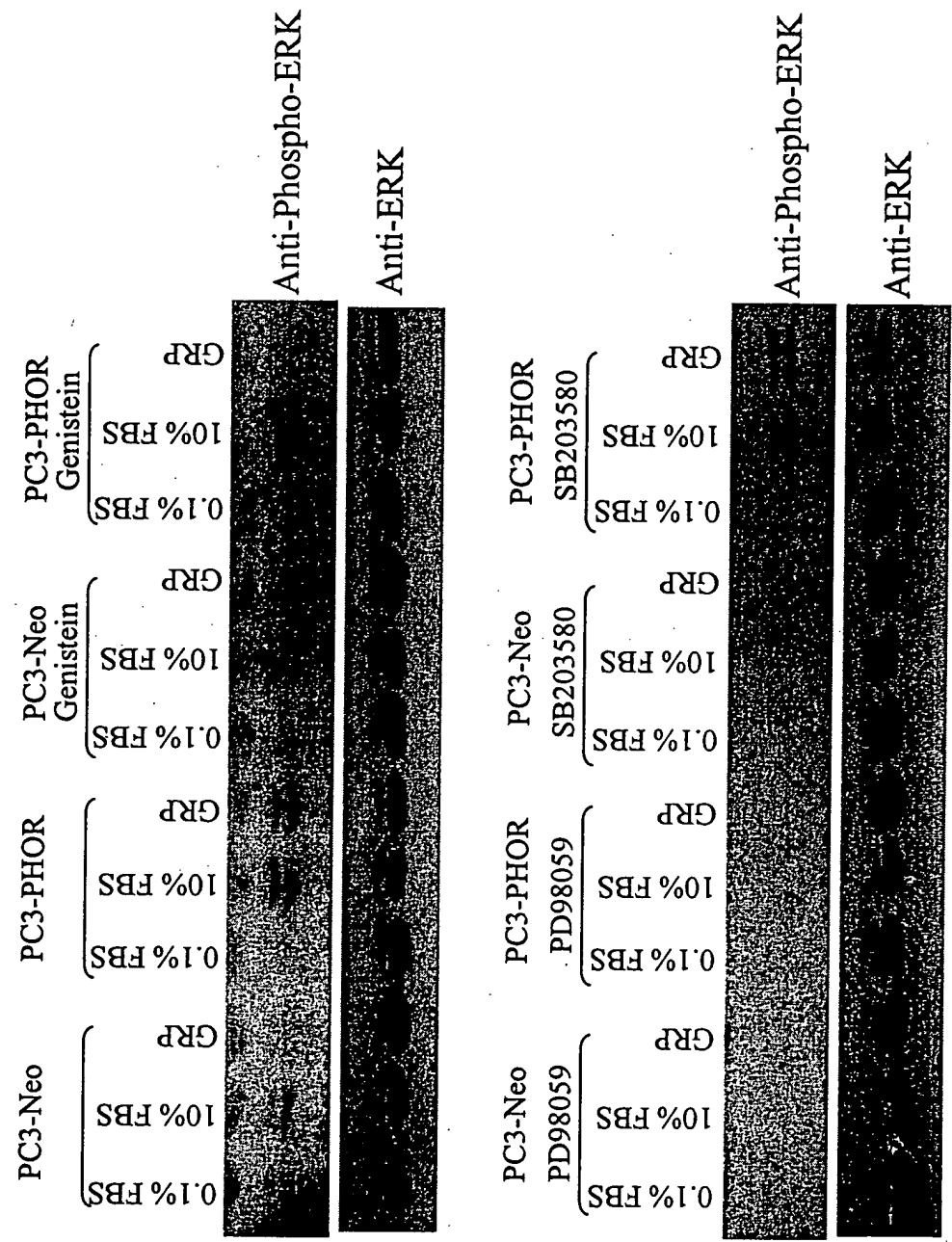
- 101P3A11 mediated the de-phosphorylation of proteins at 200, 120-140, 85-90 and 55 kDa
- 101P3A11 induced the phosphorylation of proteins at 80 and 29 kDa in NIH-3T3 cells.

Figures 27A-27B: ERK Phosphorylation by PCR Ligands in 101P3A11
Expressing Cells



•FBS, lipophosphatidic acid, gastrin releasing peptide, leukotriene and platelet activating factor induced the phosphorylation of ERK in 101P3A11 expressing cells.

Figure 28: Inhibition of 101P3A11-Mediated ERK Activation by PD98059

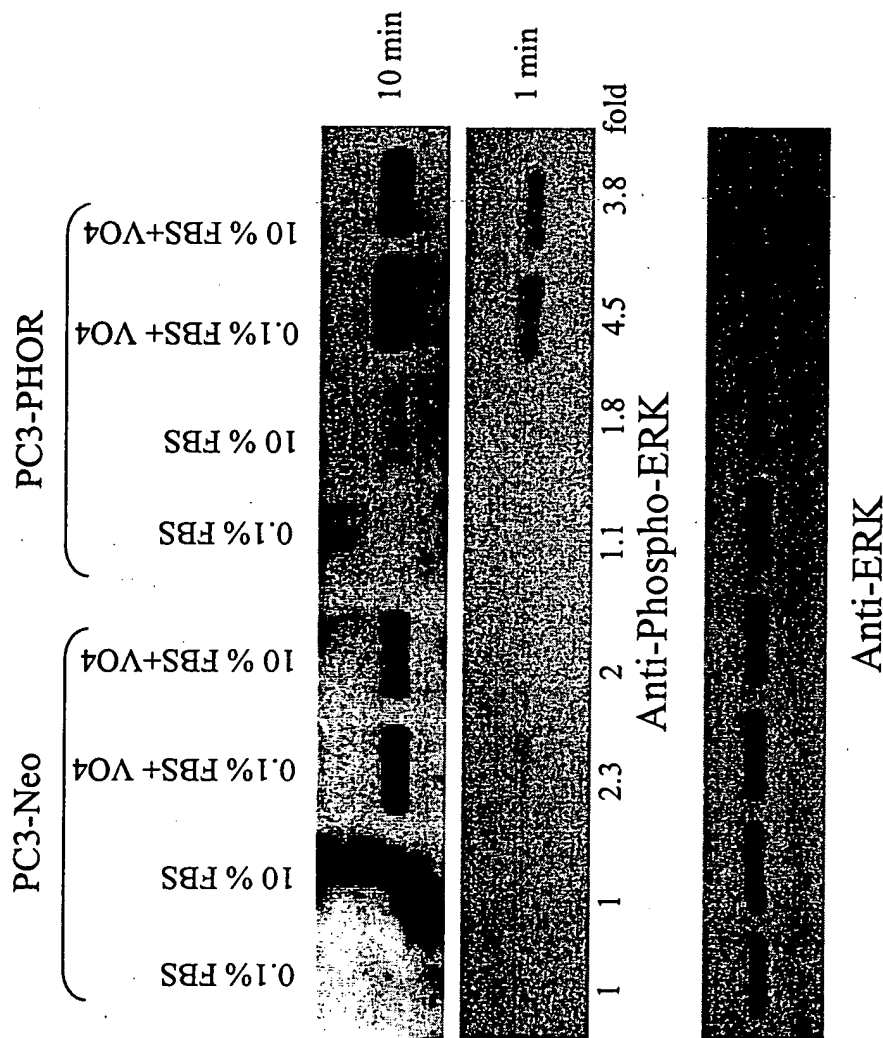


•ERK phosphorylation was inhibited by a MEK specific(PD98059) but not a p38 specific (SB203580) inhibitor in PC3-101P3A11 cells.

200250 . 6941001

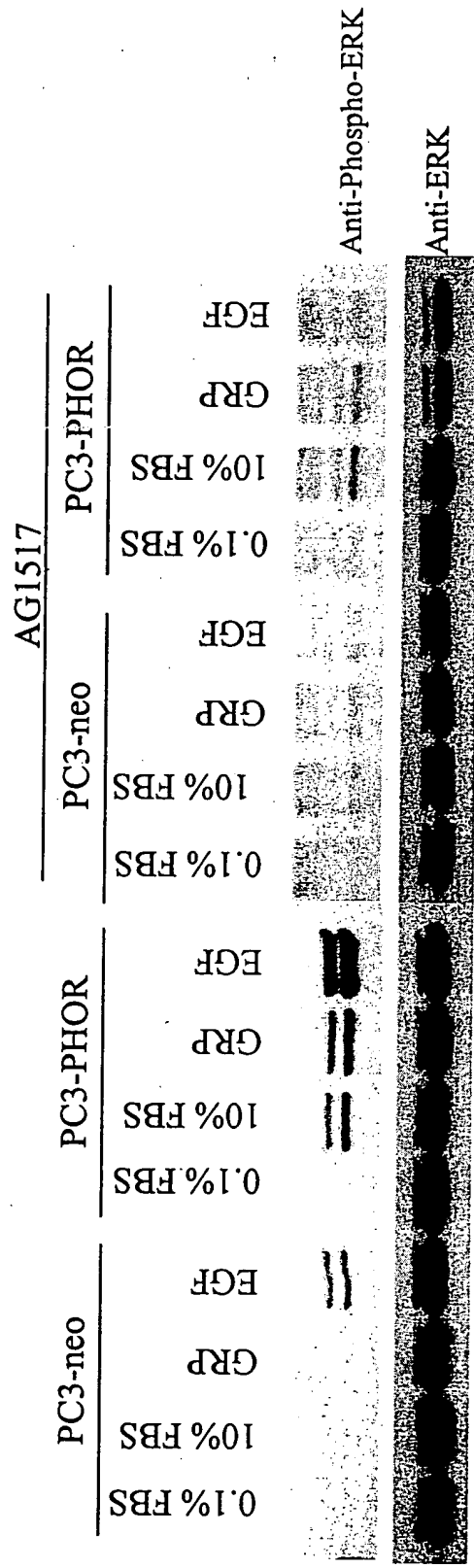


Figure 29: Enhanced ERK Phosphorylation in Sodium Orthovanadate Treated PC3-101P3A11 Cells



•Sodium orthovanadate induced increased ERK phosphorylation in PC3-101P3A11 cells relative to PC3-neo cells.

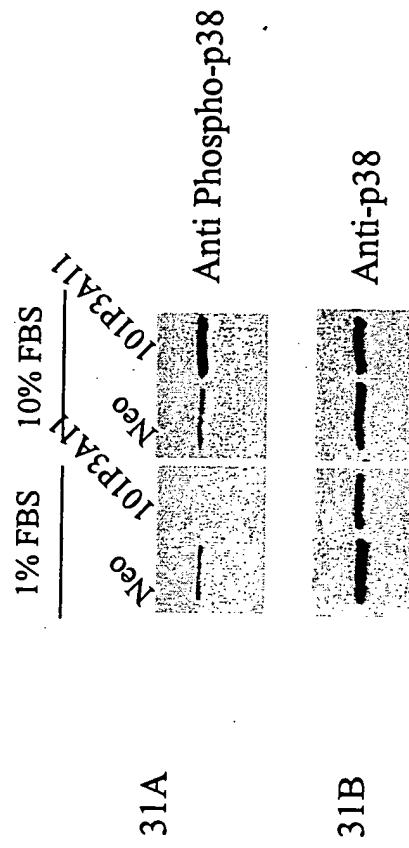
Figure 30: Inhibition of 101P3A11-Mediated ERK Phosphorylation
by AG1517



- The EGFR inhibitor, AG1517, inhibits EGF-mediated ERK phosphorylation in control and 101P3A11 expressing PC3 cells.
- AG1517 partially inhibits 101P3A11 mediated ERK phosphorylation in PC3 cells.



Figure 31A-31B: Activation of p38 in PC3-101P3A11 Cells



•Expression of 101P3A11 mediates p38 phosphorylation in cells treated with 10% FBS.



Figure 32: 101P3A11 Induced Accumulation of cAMP in PC3 Cells

Fold change in [cAMP]			
	PC3-Neo		PC3-PHOR
0.1%FBS	-PTX	1	4.302
	+PTX	1.403	2.577
10%FBS	-PTX	2.738	6.978
	+PTX	2.163	2.752

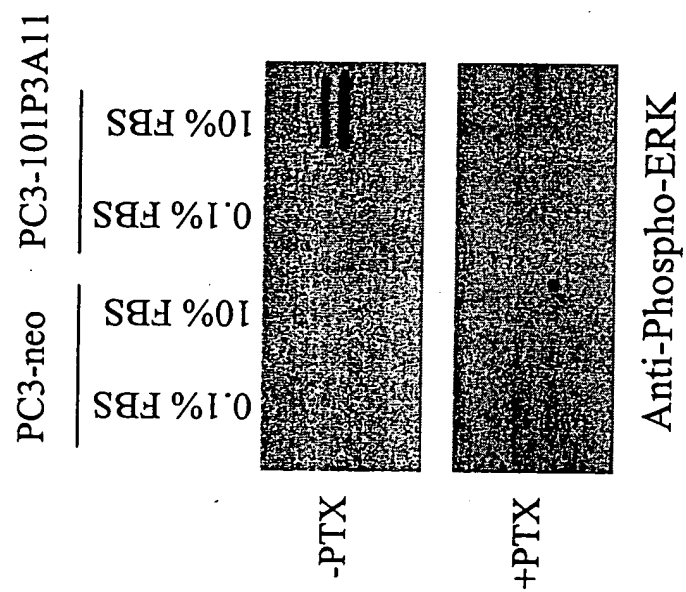
Fold Change in cAMP accumulation was calculated relative to PC3-neo cells grown in 0.1%FBS

- Expression of 101P3A11 increased the accumulation of cAMP in cells treated with 0.1% and 10% FBS.
- FBS-induced cAMP accumulation in 101P3A11 cells was inhibited by pertussis toxin.

10001460.052002

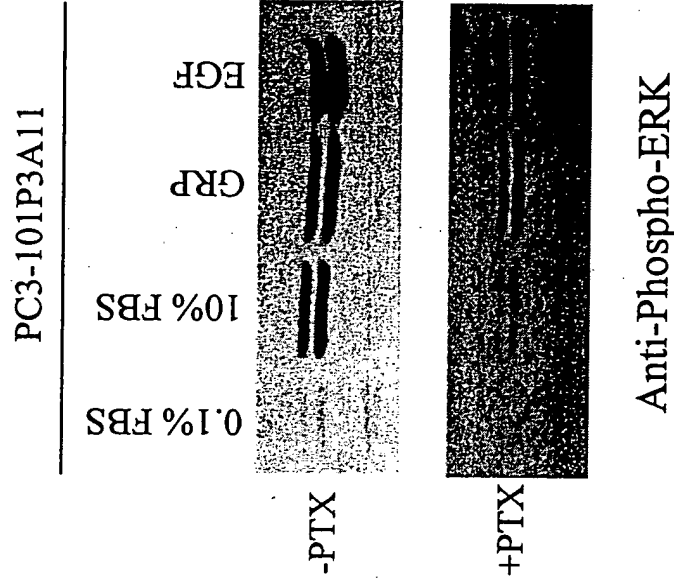


Figure 33: Pertussis Toxin Inhibits 101P3A11 Mediated ERK Phosphorylation



•Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.

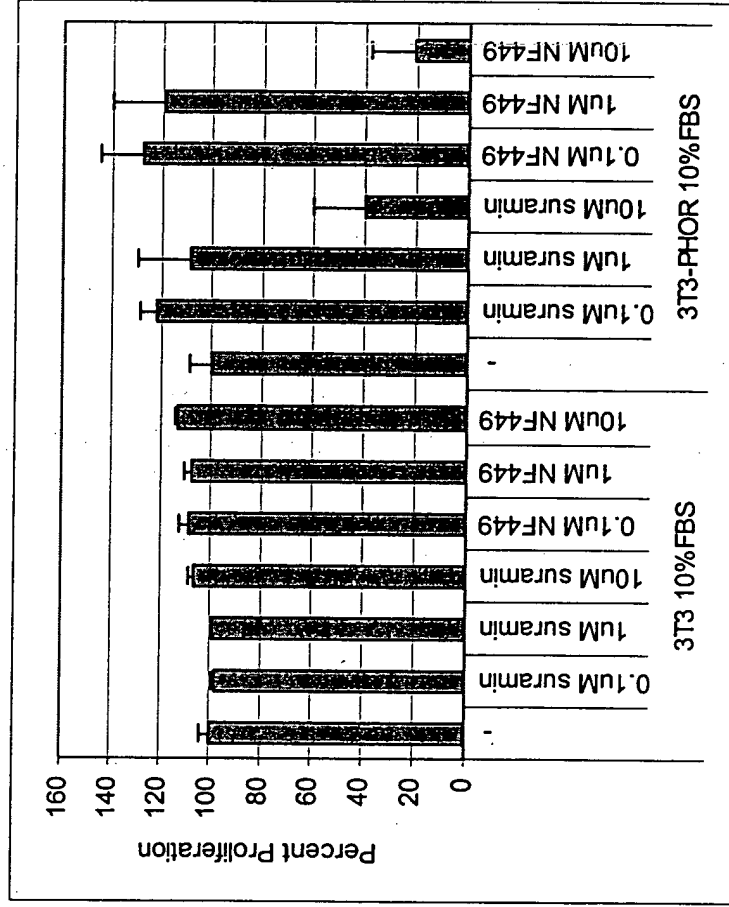
Figure 34: Pertussis Toxin Inhibited ERK Phosphorylation in PC3-101P3A11 Cells



- Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.
- The inhibitory activity of pertussis toxin on ERK phosphorylation was more dramatic in FBS-treated than EGF or GRP-treated PC3-101P3A11 cells.

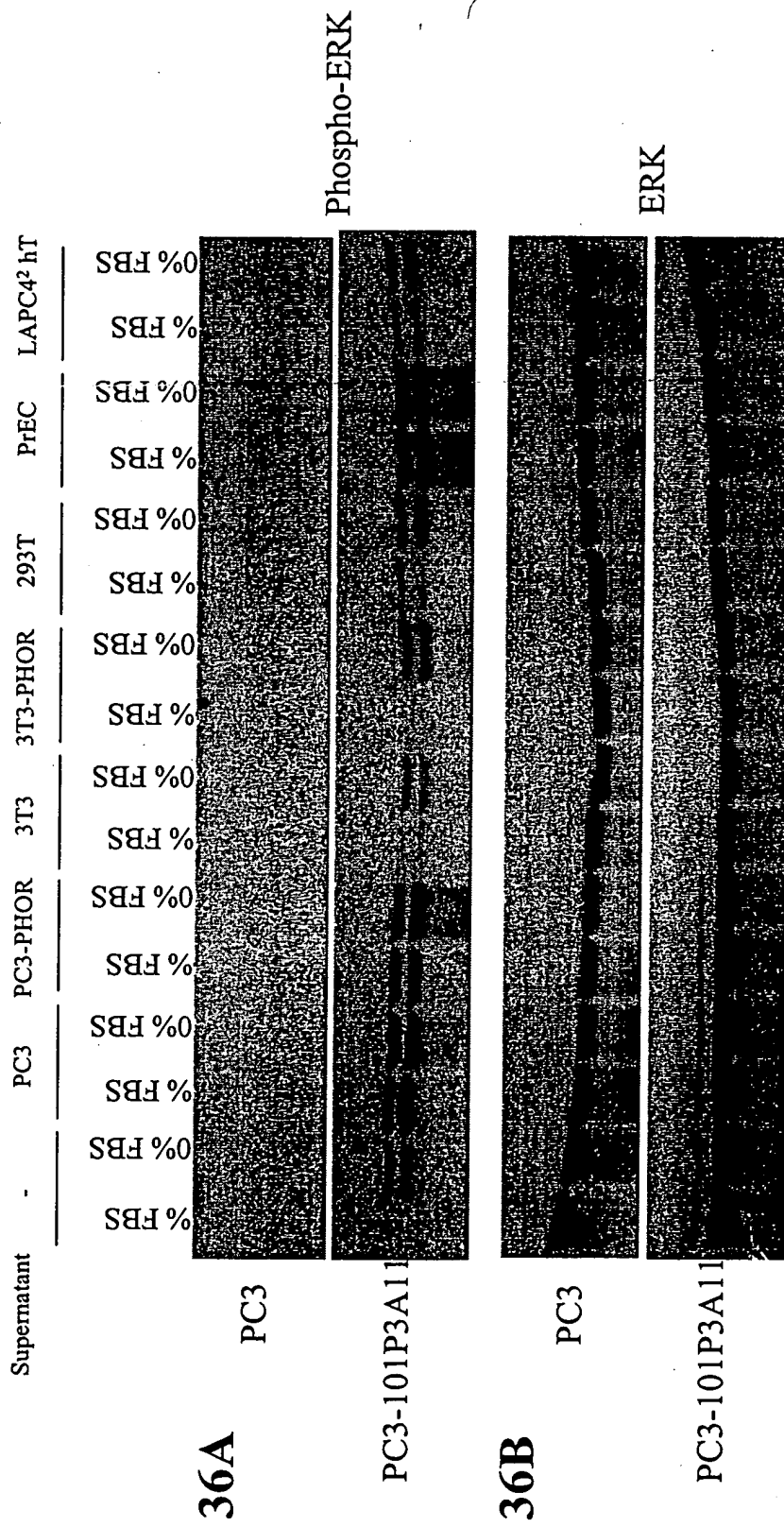


Figure 35: Inhibition of 101P3A11 Mediated Signaling by Suranim



- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence of G protein inhibitors suranim and NF449. Proliferation was analyzed by Alamar blue after 72 hours.
- Suranim and NF449 inhibited the proliferation of 101P3A11 expressing but not control cells.

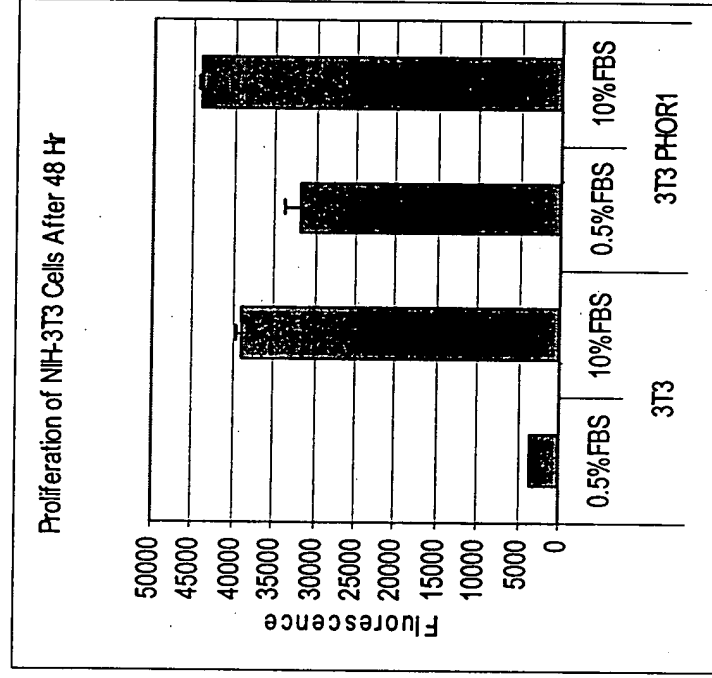
Figures 36A-36B: 101P3A11 Mediated ERK Phosphorylation By
Conditioned Media



- Supernatants from PC3, PC3-101P3A11, PrEC and LAPC4² cells induce ERK phosphorylation in PC3 101P3A11 but not PC3 cells.
- Supernatants from 3T3 and 293T cells had little specific effect on ERK phosphorylation.



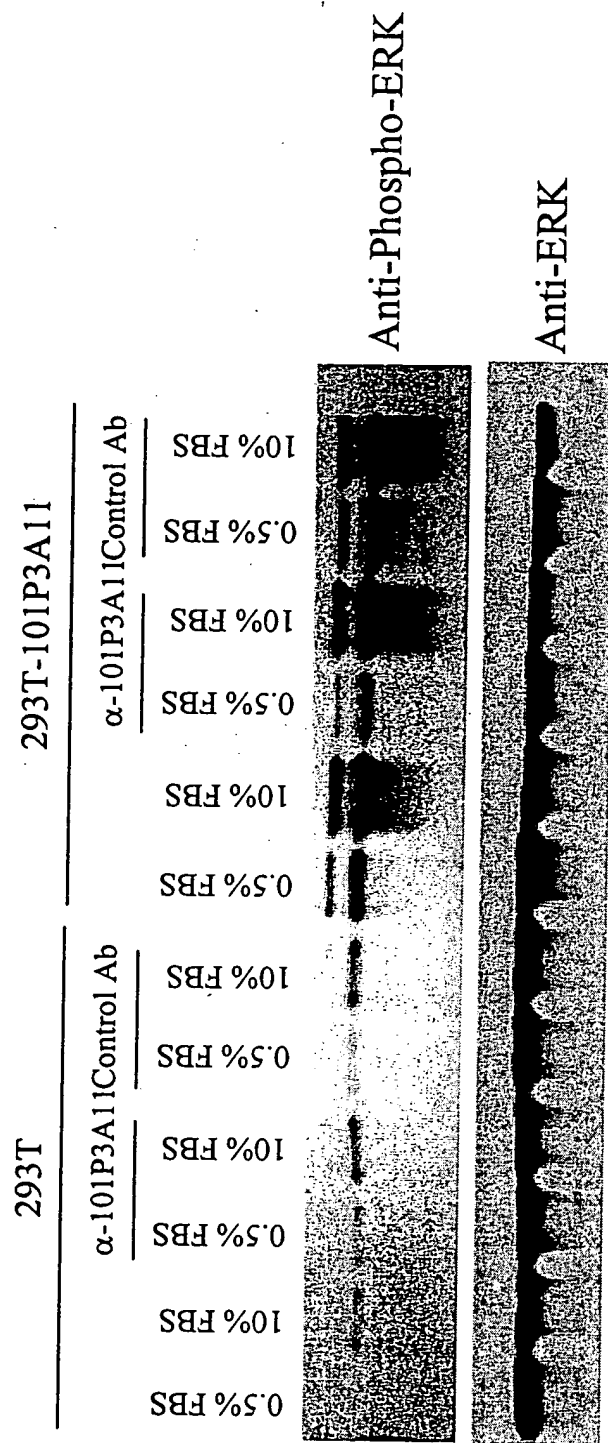
Figure 37: 101P3A11 Enhances The Proliferation of 3T3 Cells



- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence 0.5 or 10% FBS. Proliferation was analyzed by Alamar blue after 48 hours.
- Expression of 101P3A11 induced a 6 fold increase in the proliferation of 3T3 cells grown in 0.5% FBS.



Figure 38: Inhibition of 101P3A11 Mediated ERK Phosphorylation by
101P3A11 Specific Antibodies



- Expression of 101P3A11 induced ERK phosphorylation in 293T cells.
- Anti-101P3A11 pAb inhibited ERK Phosphorylation in 293T-101P3A11 cells .

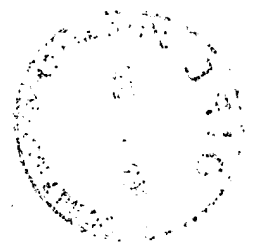
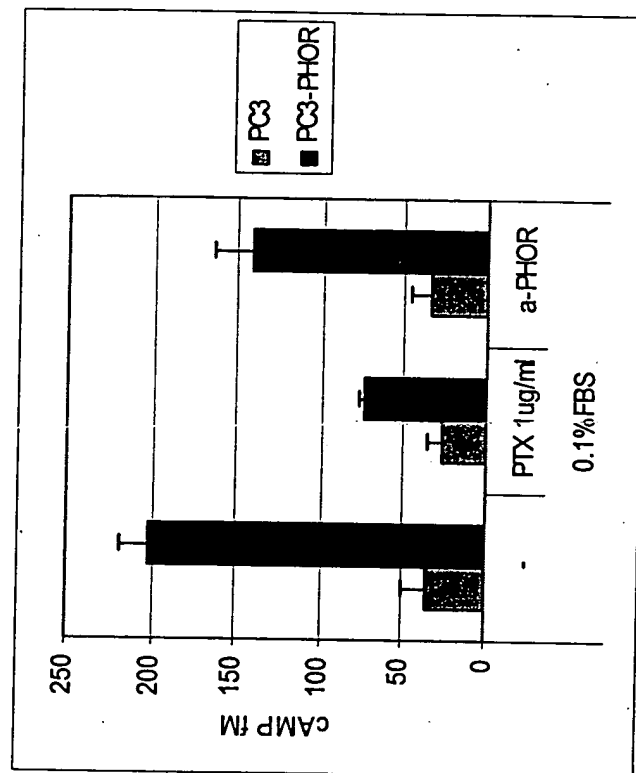


Figure 39: Anti-101P3A11 Ab Mediated cAMP Accumulation in PC3-101P3A11 Cells



Treatment	Fold Increase in cAMP	
	PC3	PC3-PHOR
0.1% FBS	1 ± 0.42	5.73 ± 0.47
PTX 1ug/ml	0.74 ± 0.28	2.12 ± 0.09
anti-PHOR	0.97 ± 0.35	4.01 ± 0.64

- Control PC3 cells and cells expressing 101P3A11 were treated with anti-101P3A11 pAb for 2 min and evaluated for intracellular cAMP content.
- The assay was performed in duplicate.

Figure 40A-40F

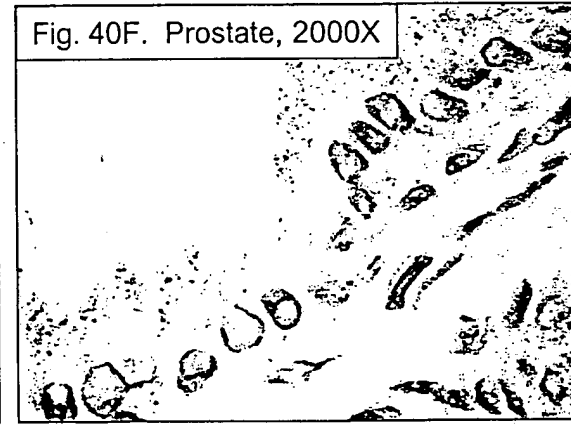
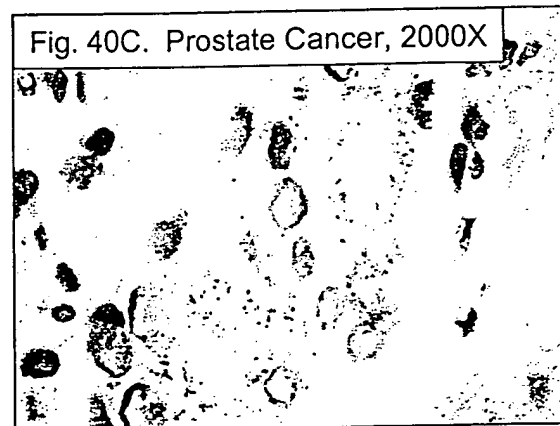
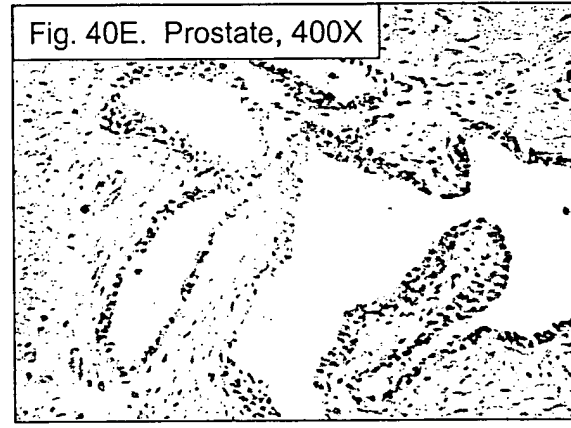
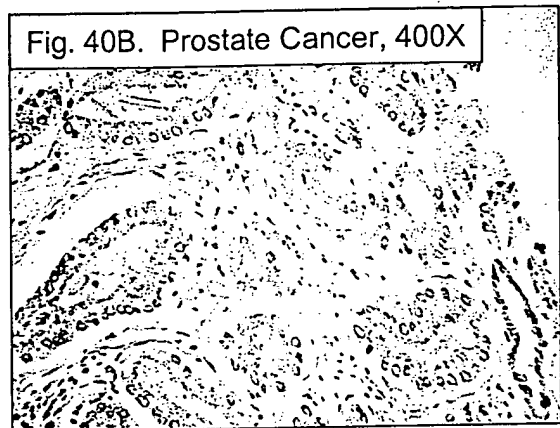
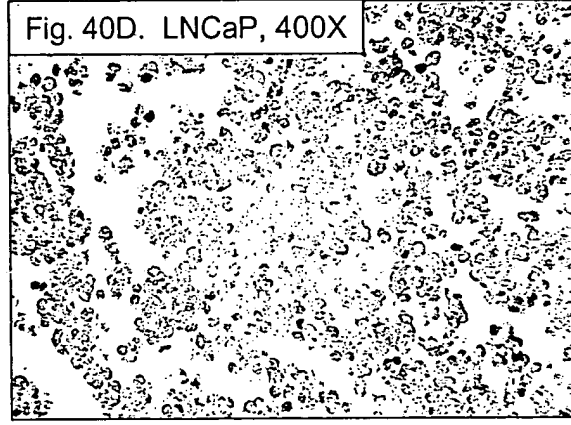
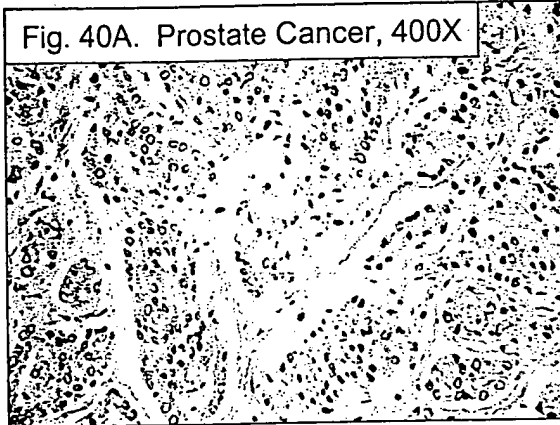


Fig.41A Prostate Cancer, 800X

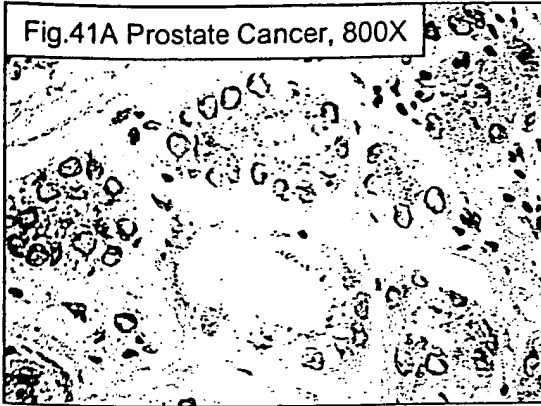


Fig.41B Bladder Cancer, 800X

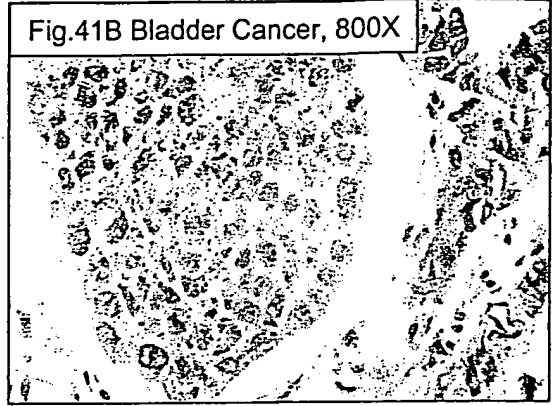


Fig.41C Kidney Cancer, 800X

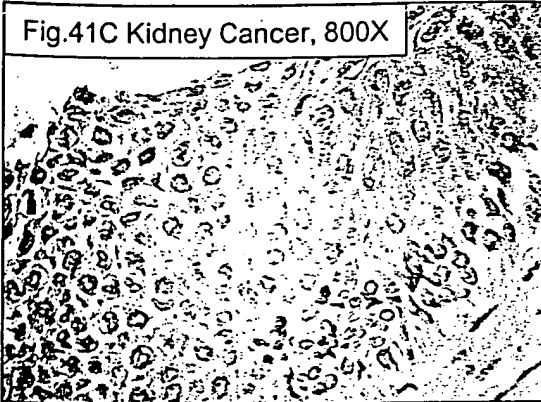


Fig.41D Colon Cancer, 800X

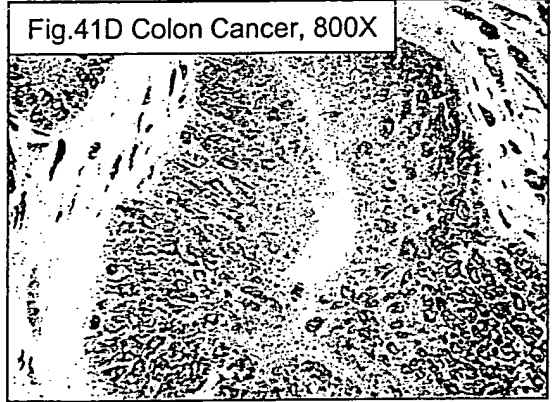


Fig.41E Lung Cancer, 800X

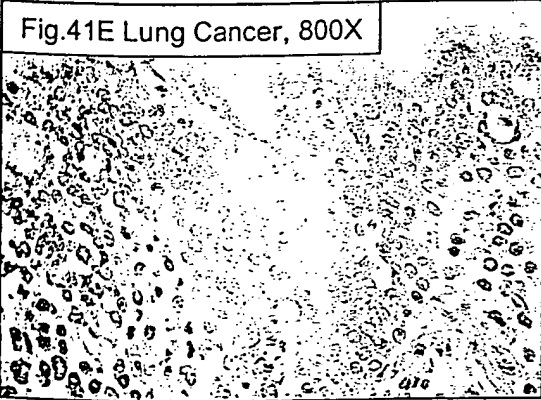


Fig.41F Breast Cancer, 800X

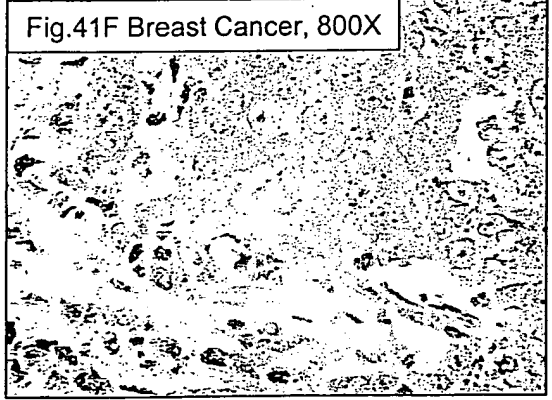


Figure 42

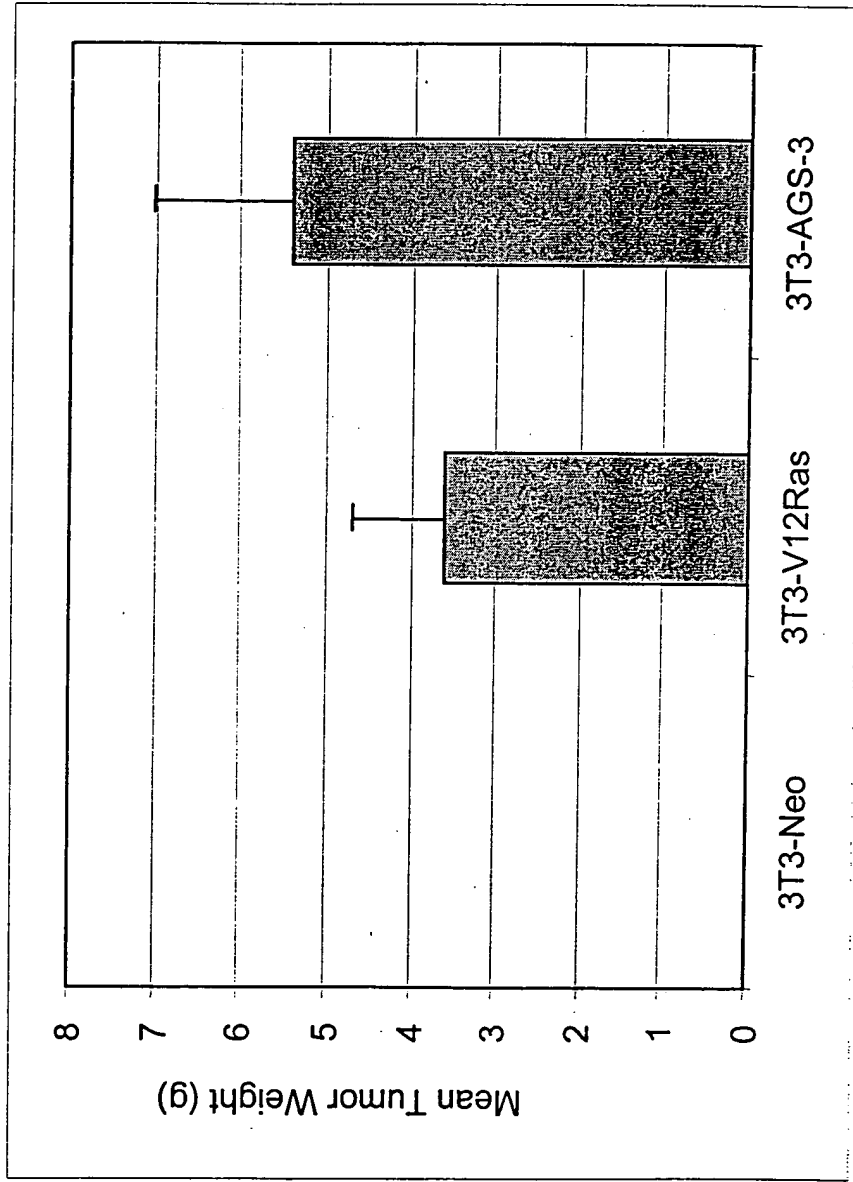
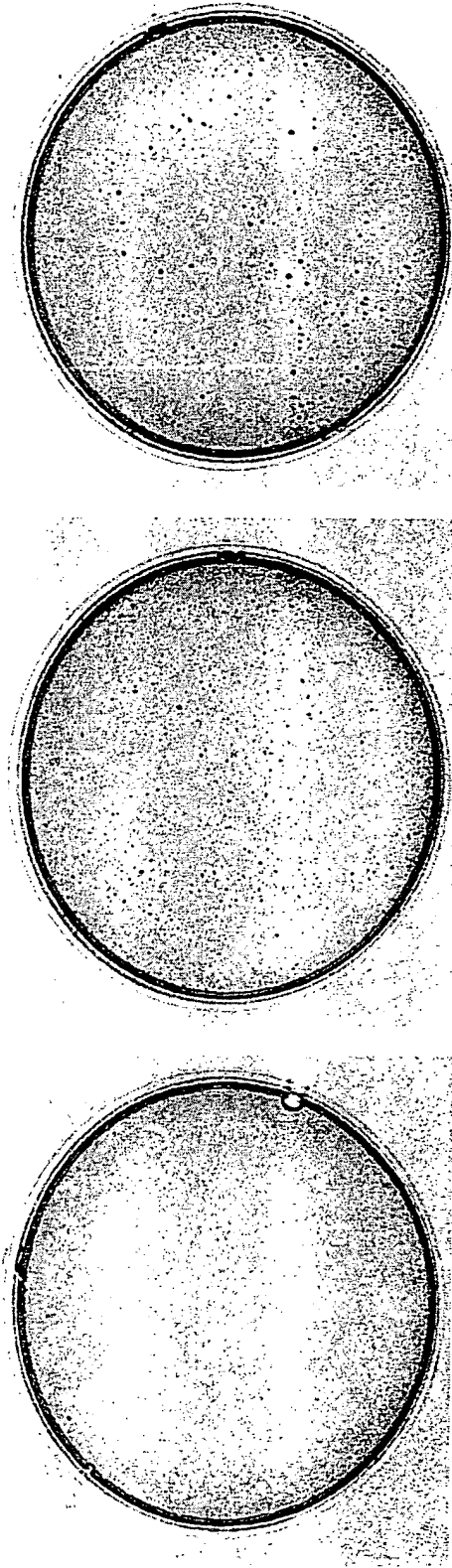


Figure 43



Neo

101P3A11

Ras

Cell Line	Colony number	
	Average	
3T3-neo	0.5	
3T3-101P3A11	686	
3T3-Ras	249	

2000250.6941001

